bioeksen
MOLECULAR DIAGNOSTICS

# LETTER OF AUTHORIZATION

By this means, the manufacturer Bioeksen AR GE Teknolojileri A.Ş, located in Huzur Mah. Metin Oktay Cad. Nurol Life Sitesi No:3/31 Sarıyer - İstanbul, authorizes the company SRL SANMEDICO to have a registered office at A. Corobceanu Street 7A, apt. 9, Chişinău MD-2012, Moldova.

As our representative and distributor carry out the necessary procedures in Moldova for the registration, importation, distribution, sales, and promotion of the products manufactured and/or assembled by Bioeksen AR GE Teknolojileri A.Ş in the Country of Moldova.

This authorization is valid for 1 year from the date of signature.

Name: Canan Z. Kotte

Firm: Bioeksen AR GE Teknolojileri A. Ş

Date: 3.02.2023

Position: Executive Manager

BİOEKSEN AR GE TEKNOLOJİLERİ A.Ş.
Huzur Mah. Metin Oktay Cad. Nurol Life D Blok
No: 3/31 Sarıyer / İSTANBUL
Maslak V.D. 176 093 2853 Tic. Sicil No: 904277-0
Mersis No: 0176 0932 8530 0001
info@bioeksen.com.tr - www.bioeksen.com.tr

Digitally signed by Monastîrschii Viorica
Date: 2023.03.20 17:00:53 EET
Reason: MoldSign Signature
Location: Moldova

Tel. : +90 (212) 285 10 17
Fax: +90 (212) 285 10 18

www.bioeksen.com.tr
info@bioeksen.com.tr

Huzur Mah. Metin Oktay Cad. Nurol Life Sitesi
D Blok No:3/31 Sarıyer-Istanbul-TURKEY

# EC DECLARATION OF CONFORMITY

## Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Medical Diagnostic Devices

**Bioeksen AR GE Teknolojileri A.Ş.** hereby declares under its own responsibility that the products covered by this declaration conform with "Essential Requirements" listed in Annex I of EC Directive 98/79/EC (IVD Directive). Supporting documentation (technical documentation) is retained under the premises of the manufacturer.

| | |
|---|---|
| **Manufacturer** | : Bioeksen AR GE Teknolojileri Anonim Şirketi |
| Central Office | : Huzur Mah. Metin Oktay Cad. Nurol Life Sitesi D Blok No:3/31, 34396 Sarıyer/İstanbul TÜRKİYE |
| Manufacturing Site | : Huzur Mahallesi Metin Oktay Caddesi Nurol Life No:3/10, Sarıyer/İstanbul TÜRKİYE |
| | Web: www.bioeksen.com.tr, E-mail: info@bioeksen.com.tr |
| **Product(s) Name** | : Bio-Speedy® Bordetella pertussis, B.parapertussis, B.bronchiseptica and B.holmesii Real-Time PCR Detection Kit |
| **Description** | : Bio-Speedy® Bordetella pertussis, B.parapertussis, B.bronchiseptica and B.holmesii Real-Time PCR Detection Kit |
| | Ref No: BS-DTC-103-25 |
| | Ref No: BS-DTC-103-100 |
| **Classification** | : Other (Neither listed in the Annex II, Nor Self-testing device), GMDN code: 50505 - Multiple Bordetella species nucleic acid IVD, kit, nucleic acid technique (NAT) |
| | Article 9, paragraph 1 of EC Council Directive |
| | 98/79/EC on In Vitro Medical Diagnostic Devices |
| **Conformity Assessment Route** | : According to Annex III of the IVD Directive 98/79/EC |
| | EC declaration of conformity under manufacturer responsibility |
| **Applied Standards** | : All standards stated in the annex on the other page are strictly |
| | implemented in our company. |

We hereby declare that the above-mentioned product/s meet the provisions of the EC Council Directive 98/79/EC for in vitro medical diagnostic devices. All supporting documentation is retained under the premises of the manufacturer and the manufacturer is exclusively responsible for the declaration of conformity.

BİOEKSEN AR GE TEKNOLOJİLERİ A.Ş.
Huzur Mah. Metin Oktay Cad. Nurol Life D Blok
No: 3/31 Sarıyer / İSTANBUL
Maslak V.D. 176 093 2853 Tic. Sicil No: 904277-0
Mersis No: 0176 0932 8530 0001
info@bioeksen.com.tr - www.bioeksen.com.tr

***Signature:***

**Place of Issue:** İstanbul

***Valid from:*** 25.05.2022

**Authorized Person:** Canan Zöhre Ketre Kolukırık

*Chairman of the Board*

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CE IVD

# EC DECLARATION OF CONFORMITY

**Attachment**
**List of Applied Standards**

| No. | Title of standards | Contents |
|---|---|---|
| 1 | EN ISO 13485:2016 | Medical devices - Quality management systems - Requirements for regulatory purposes |
| 2 | EN ISO 14971:2019 | Medical devices – Application of risk management to medical devices |
| 3 | EN ISO 17511:2020 | In vitro diagnostic medical devices - Measurement of quantities in biological samples - Metrological traceability of values assigned to calibrators and control materials |
| 4 | EN 13612:2002 | Performance evaluation of in vitro diagnostic medical devices |
| 5 | EN ISO 23640:2015 | In vitro diagnostic medical devices – Evaluation of stability of in vitro diagnostic reagents |
| 6 | EN ISO 18113-1:2011 | In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 1: Terms, definitions, and general requirements |
| 7 | EN ISO 18113-2:2011 | In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 2: In vitro diagnostic reagents for professional use |
| 8 | EN ISO 15223-1:2021 | Medical devices - Symbols to be used with medical device labels, labelling and information to be supplied - Part 1: General requirements |
| 9 | IEC 62366-1:2015 | Medical devices — Part 1: Application of usability engineering to medical devices |
| 10 | CLSI MM3 A3: 3ED 2015 | Molecular Diagnostic Methods for Infectious Diseases |
| 11 | CLSI EP17 A2: 2ED 2012 | Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures |
| 12 | CLSI EP07 3ED: 2018 | Interference Testing in Clinical Chemistry, 3rd Edition |
| 13 | CLSI EP5 A3: 3ED 2014 | Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition |

# EC DECLARATION OF CONFORMITY

## Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Medical Diagnostic Devices

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|---|---|
| **Manufacturer** | : Bioeksen AR GE Teknolojileri Anonim Şirketi<br>Huzur Mah. Metin Oktay Cad. Nurol Life Sitesi D Blok No:3/31, 34396, Sarıyer/İstanbul TÜRKİYE<br>Web: www.bioeksen.com.tr, e-mail: info@bioeksen.com.tr |
| **Product(s) Name** | : Bio-Speedy® Bacillus anthracis Real-Time PCR Detection Kit |
| **Description** | : Bio-Speedy® Bacillus anthracis Real-Time PCR Detection Kit<br>Ref No: BS-DTC-V-224-25<br>Ref No: BS-DTC-V-224-100 |
| **Classification** | : Other (Neither listed in the Annex II, Nor Self-testing device), GMDN code: 50432 - Bacillus anthracis nucleic acid IVD, kit, nucleic acid technique (NAT)<br>Article 9, paragraph 1 of EC Council Directive<br>98/79/EC on In Vitro Medical Diagnostic Devices |
| **Conformity Assessment Route** | : According to Annex III of the IVD Directive 98/79/EC<br>EC declaration of conformity under manufacturer responsibility |
| **Applied Standards** | : All standards stated in the annex on the other page are strictly implemented in our company. |

We hereby declare that the above-mentioned product/s meet the provisions of the EC Council Directive 98/79/EC for in vitro medical diagnostic devices. All supporting documentation is retained under the premises of the manufacturer and the manufacturer is exclusively responsible for the declaration of conformity.

**Place of Issue:** İstanbul

***Valid from:*** 25.05.2022

***Signature:***

BİOEKSEN AR GE TEKNOLOJİLERİ A.Ş.
Huzur Mah. Metin Oktay Cad. Nurol Life D Blok
No: 3/31 Sarıyer / İSTANBUL
Maslak V.D. 176 093 2853 Ticaret Sicil No: 904277-0
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**Authorized Person:** Canan Zöhre Ketre Kolukırık

*Chairman of the Board*

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CE IVD

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| 5 | EN ISO 23640:2015 | In vitro diagnostic medical devices – Evaluation of stability of in vitro diagnostic reagents |
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| **Manufacturer** | : Bioeksen AR GE Teknolojileri Anonim Şirketi |
| Central Office | : Huzur Mah. Metin Oktay Cad. Nurol Life Sitesi D Blok No:3/31, 34396 Sarıyer/İstanbul TÜRKİYE |
| Manufacturing Site | : Huzur Mahallesi Metin Oktay Caddesi Nurol Life No:3/10, Sarıyer/İstanbul TÜRKİYE |
| | Web: www.bioeksen.com.tr, E-mail: info@bioeksen.com.tr |
| **Product(s) Name** | : Bio-Speedy® Bacillus anthracis Real-Time PCR Detection Kit |
| **Description** | : Bio-Speedy® Bacillus anthracis Real-Time PCR Detection Kit |
| | Ref No: BS-DTC-V-224-25 |
| | Ref No: BS-DTC-V-224-100 |
| **Classification** | : Other (Neither listed in the Annex II, Nor Self-testing device), GMDN code: 50432 - Bacillus anthracis nucleic acid IVD, kit, nucleic acid technique (NAT) |
| | Article 9, paragraph 1 of EC Council Directive |
| | 98/79/EC on In Vitro Medical Diagnostic Devices |
| **Conformity Assessment Route** | : According to Annex III of the IVD Directive 98/79/EC |
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We hereby declare that the above-mentioned product/s meet the provisions of the EC Council Directive 98/79/EC for in vitro medical diagnostic devices. All supporting documentation is retained under the premises of the manufacturer and the manufacturer is exclusively responsible for the declaration of conformity.

***Signature:***

BİOEKSEN AR GE TEKNOLOJİLERİ A.Ş.
Huzur Mah. Metin Oktay Cad. Nurol Life D Blok
No: 3/31 Sarıyer / İSTANBUL
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info@bioeksen.com.tr - www.bioeksen.com.tr

**Place of Issue:** İstanbul

***Valid from:*** 25.05.2022

**Authorized Person:** Canan Zöhre Ketre Kolukırık

*Chairman of the Board*

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# EC DECLARATION OF CONFORMITY

**Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Medical Diagnostic Devices**

**Bioeksen AR GE Teknolojileri A.Ş.** hereby declares under its own responsibility that the products covered by this declaration conform with "Essential Requirements" listed in Annex I of EC Directive 98/79/EC (IVD Directive). Supporting documentation (technical documentation) is retained under the premises of the manufacturer.

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|---|---|
| **Manufacturer** | : Bioeksen AR GE Teknolojileri Anonim Şirketi<br>Huzur Mah. Metin Oktay Cad. Nurol Life Sitesi D Blok No:3/31, 34396, Sarıyer/İstanbul TÜRKİYE<br>Web: www.bioeksen.com.tr, e-mail: info@bioeksen.com.tr |
| **Product(s) Name** | : Bio-Speedy® Measles Virus Real-Time PCR Detection Kit |
| **Description** | : Bio-Speedy® Measles Virus Real-Time PCR Detection Kit<br>Ref No: BS-MEV-DTC-320-25<br>Ref No: BS-MEV-DTC-320-100 |
| **Classification** | : Other (Neither listed in the Annex II, Nor Self-testing device), GMDN code: 49276 - Measles virus nucleic acid IVD, kit, nucleic acid technique (NAT)<br>Article 9, paragraph 1 of EC Council Directive<br>98/79/EC on In Vitro Medical Diagnostic Devices |
| **Conformity Assessment Route** | : According to Annex III of the IVD Directive 98/79/EC<br>EC declaration of conformity under manufacturer responsibility |
| **Applied Standards** | : All standards stated in the annex on the other page are strictly implemented in our company. |

We hereby declare that the above-mentioned product/s meet the provisions of the EC Council Directive 98/79/EC for in vitro medical diagnostic devices. All supporting documentation is retained under the premises of the manufacturer and the manufacturer is exclusively responsible for the declaration of conformity.

**Place of Issue:** İstanbul

***Valid from:*** 25.05.2022

***Signature:***

BİOEKSEN AR GE TEKNOLOJİLERİ A.Ş.
Huzur Mah. Metin Oktay Cad. Nurol Life D Blok
No: 3/31 Sarıyer / İSTANBUL
Maslak V.D. 176 093 2658 Tic. Sicil No: 904277-0
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**Authorized Person:** Canan Zöhre Ketre Kolukırık

*Chairman of the Board*

bioeksen

CE IVD

# EC DECLARATION OF CONFORMITY

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**List of Applied Standards**

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| 5 | EN ISO 23640:2015 | In vitro diagnostic medical devices – Evaluation of stability of in vitro diagnostic reagents |
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| 7 | EN ISO 18113-2:2011 | In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 2: In vitro diagnostic reagents for professional use |
| 8 | EN ISO 15223-1:2021 | Medical devices - Symbols to be used with medical device labels, labelling and information to be supplied - Part 1: General requirements |
| 9 | IEC 62366-1:2015 | Medical devices — Part 1: Application of usability engineering to medical devices |
| 10 | CLSI MM3 A3: 3ED 2015 | Molecular Diagnostic Methods for Infectious Diseases |
| 11 | CLSI EP17 A2: 2ED 2012 | Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures |
| 12 | CLSI EP07 3ED: 2018 | Interference Testing in Clinical Chemistry, 3rd Edition |
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|---|---|
| **Manufacturer** | : Bioeksen AR GE Teknolojileri Anonim Şirketi |
| Central Office | : Huzur Mah. Metin Oktay Cad. Nurol Life Sitesi D Blok No:3/31, 34396 Sarıyer/İstanbul TÜRKİYE |
| Manufacturing Site | : Huzur Mahallesi Metin Oktay Caddesi Nurol Life No:3/10, Sarıyer/İstanbul TÜRKİYE |
| | Web: www.bioeksen.com.tr, E-mail: info@bioeksen.com.tr |
| **Product(s) Name** | : Bio-Speedy® West Nile Virus Real-Time PCR Detection Kit |
| **Description** | : Bio-Speedy® West Nile Virus Real-Time PCR Detection Kit |
| | Ref No: BS-BNV-DTC-322-25 |
| | Ref No: BS-BNV-DTC-322-100 |
| **Classification** | : Other (Neither listed in the Annex II, Nor Self-testing device), GMDN code: 49045 - West Nile virus nucleic acid IVD, kit, nucleic acid technique (NAT) |
| | Article 9, paragraph 1 of EC Council Directive |
| | 98/79/EC on In Vitro Medical Diagnostic Devices |
| **Conformity Assessment Route** | : According to Annex III of the IVD Directive 98/79/EC |
| | EC declaration of conformity under manufacturer responsibility |
| **Applied Standards** | : All standards stated in the annex on the other page are strictly |
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We hereby declare that the above-mentioned product/s meet the provisions of the EC Council Directive 98/79/EC for in vitro medical diagnostic devices. All supporting documentation is retained under the premises of the manufacturer and the manufacturer is exclusively responsible for the declaration of conformity.

**Place of Issue:** İstanbul

***Valid from:*** 25.05.2022

***Signature:***

BİOEKSEN AR GE TEKNOLOJİLERİ A.Ş.
Huzur Mah. Metin Oktay Cad. Nurol Life D Blok
No: 3/31 Sarıyer / İSTANBUL
Maslak V.D. 176 093 2853 Tic. Sicil No: 904277-0
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**Authorized Person:** Canan Zöhre Ketre Kolukırık

*Chairman of the Board*

# EC DECLARATION OF CONFORMITY

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**List of Applied Standards**

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| 10 | CLSI MM3 A3: 3ED 2015 | Molecular Diagnostic Methods for Infectious Diseases |
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| **Manufacturer** | : Bioeksen AR GE Teknolojileri Anonim Şirketi |
| Central Office | : Huzur Mah. Metin Oktay Cad. Nurol Life Sitesi D Blok No:3/31, 34396 Sarıyer/İstanbul TÜRKİYE |
| Manufacturing Site | : Huzur Mahallesi Metin Oktay Caddesi Nurol Life No:3/10, Sarıyer/İstanbul TÜRKİYE |
| | Web: www.bioeksen.com.tr, E-mail: info@bioeksen.com.tr |
| **Product(s) Name** | : Bio-Speedy® CCHFV RT-qPCR Detection Kit |
| **Description** | : Bio-Speedy® CCHFV RT-qPCR Detection Kit |
| | Ref No: CCHFVD0125 |
| | Ref No: CCHFVD01100 |
| **Classification** | : Other (Neither listed in the Annex II, Nor Self-testing device), GMDN code: 49916 - Crimean-Congo hemorrhagic fever (CCHF) virus nucleic acid IVD, kit, nucleic acid technique (NAT) |
| | Article 9, paragraph 1 of EC Council Directive |
| | 98/79/EC on In Vitro Medical Diagnostic Devices |
| **Conformity Assessment Route** | : According to Annex III of the IVD Directive 98/79/EC |
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***Signature:***

BİOEKSEN AR GE TEKNOLOJİLERİ A.Ş.
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**Place of Issue:** İstanbul

***Valid from:*** 25.05.2022

**Authorized Person:** Canan Zöhre Ketre Kolukırık

*Chairman of the Board*

bioeksen

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**Bioeksen R&D Technologies Inc. Co.** hereby declares under its own responsibility that the products covered by this declaration conform with "Essential Requirements" listed in Annex I of EC Directive 98/79/EC (IVD Directive). Supporting documentation (technical documentation) is retained under the premises of the manufacturer.

| | |
|---|---|
| **Manufacturer** | : Bioeksen R&D Technologies Incorporated Company<br>Resitpasa Mh. Katar Cd. No:4/B-105. Sariyer, Istanbul- TURKEY<br>Web: www.bioeksen.com.tr, e-mail: info@bioeksen.com.tr |
| **Product(s) Name** | : Bio-Speedy® Legionella pneumophila qPCR Kit |
| **Description** | : Bio-Speedy® Legionella pneumophila qPCR Kit<br>Ref No: BS-LP-25<br>Ref No: BS-LP-100 |
| **Classification** | : Other (Neither listed in the Annex II, Nor Self-testing device), GMDN code: 51060 - Legionella pneumophila nucleic acid IVD, kit, nucleic acid technique (NAT)<br>Article 9, paragraph 1 of EC Council Directive<br>98/79/EC on In Vitro Medical Diagnostic Devices |
| **Conformity Assessment Route** | : According to Annex III of the IVD Directive 98/79/EC<br>EC declaration of conformity under manufacturer responsibility |
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**Place of Issue:** Istanbul

***Valid from:*** 16.05.2022

***Signature:***

**Authorized Person:** Canan Zöhre Ketre Kolukırık

*Chairman of the Board*

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**Attachment**
**List of Applied Standards**

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| 8 | EN ISO 15223-1:2021 | Medical devices - Symbols to be used with medical device labels, labelling and information to be supplied - Part 1: General requirements |
| 9 | IEC 62366-1:2015 | Medical devices — Part 1: Application of usability engineering to medical devices |
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# EC DECLARATION OF CONFORMITY

**Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Medical Diagnostic Devices**

**Bioeksen R&D Technologies Inc. Co.** hereby declare under own responsibility that the products covered by this declaration conform with "Essential Requirements" listed in Annex I of EC Directive 98/79/EC (IVD Directive). Supporting documentation (technical documentation) is retained under the premises of the manufacturer.

| | |
|---|---|
| **Manufacturer** | : Bioeksen R&D Technologies Incorporated Company<br>Resitpasa Mh. Katar Cd. No:4/B-105. Sariyer, Istanbul- TURKEY<br>Web: www.bioeksen.com.tr, e-mail: info@bioeksen.com.tr |
| **Product(s) Name** | : Bio-Speedy® CCHFV RT-qPCR Detection Kit |
| **Description** | : Bio-Speedy® CCHFV RT-qPCR Detection Kit<br>Ref No: CCHFVD01100 |
| **Classification** | : Other (Neither listed in the Annex II, Nor Self-testing device), GMDN code: 49916 - Crimean-Congo hemorrhagic fever (CCHF) virus nucleic acid IVD, kit, nucleic acid technique (NAT)<br>Article 9, paragraph 1 of EC Council Directive<br>98/79/EC on In Vitro Medical Diagnostic Devices |
| **Conformity Assessment Route** | : According to Annex III of the IVD Directive 98/79/EC<br>EC declaration of conformity under manufacturer responsibility |
| **Applied Standards** | : All standards stated in the annex on the other page are strictly implemented in our company. |

We hereby declare that the above-mentioned product/s meet the provisions of the EC Council Directive 98/79/EC for in vitro medical diagnostic devices. All supporting documentation is retained under the premises of the manufacturer and manufacturer is exclusively responsible for the declaration of conformity.

Place of Issue: Istanbul

*Signature:*

*Valid from:* 25.06.2021

Authorized Person: Canan Z. KETRE KOLUKIRIK/Company Manager

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|---|---|
| **Manufacturer** | : Bioeksen R&D Technologies Incorporated Company<br>Resitpasa Mh. Katar Cd. No:4/B-105. Sariyer, Istanbul- TURKEY<br>Web: www.bioeksen.com.tr, e-mail: info@bioeksen.com.tr |
| **Product(s) Name** | : Bio-Speedy® Brucella spp. qPCR Kit |
| **Description** | : Bio-Speedy® Brucella spp. qPCR Kit<br>Ref No: BS-SP-B-12-50<br>Ref No: BS-SP-B-12-100<br>Ref No: BS-SP-B-12-250 |
| **Classification** | : Other (Neither listed in the Annex II, Nor Self-testing device), GMDN code: 50605 - Multiple Brucella species nucleic acid IVD, kit, nucleic acid technique (NAT)<br>Article 9, paragraph 1 of EC Council Directive<br>98/79/EC on In Vitro Medical Diagnostic Devices |
| **Conformity Assessment Route** | : According to Annex III of the IVD Directive 98/79/EC<br>EC declaration of conformity under manufacturer responsibility |
| **Applied Standards** | : All standards stated in the annex on the other page are strictly implemented in our company. |

We hereby declare that the above-mentioned product/s meet the provisions of the EC Council Directive 98/79/EC for in vitro medical diagnostic devices. All supporting documentation is retained under the premises of the manufacturer and manufacturer is exclusively responsible for the declaration of conformity.

**Place of Issue:** Istanbul

***Signature:***

***Valid from:*** 06.04.2022

**Authorized Person:** Begum Gizem Gokirmak

*Regulatory Affairs and Quality Manager*

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# EC DECLARATION OF CONFORMITY

**Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Medical Diagnostic Devices**

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|---|---|
| **Manufacturer** | : Bioeksen R&D Technologies Incorporated Company<br>Resitpasa Mh. Katar Cd. No:4/B-105. Sariyer, Istanbul- TURKEY<br>Web: www.bioeksen.com.tr, e-mail: info@bioeksen.com.tr |
| **Product(s) Name** | : vNAT® Transfer Tube |
| **Description** | : vNAT® Transfer Tube<br>Ref No: BS-NA-513m-100 |
| **Classification** | : Other (Neither listed in the Annex II, Nor Self-testing device), GMDN code: 62392 - Oral/respiratory tract specimen container IVD, additive/medium<br>Article 9, paragraph 1 of EC Council Directive<br>98/79/EC on In Vitro Medical Diagnostic Devices |
| **Conformity Assessment Route** | : According to Annex III of the IVD Directive 98/79/EC<br>EC declaration of conformity under manufacturer responsibility |
| **Applied Standards** | : All standards stated in the annex on the other page are strictly<br>implemented in our company. |

We hereby declare that the above-mentioned product/s meet the provisions of the EC Council Directive 98/79/EC for in vitro medical diagnostic devices. All supporting documentation is retained under the premises of the manufacturer and manufacturer is exclusively responsible for the declaration of conformity.

**Place of Issue:** Istanbul

***Signature:***

***Valid from:*** 28.02.2022

**Authorized Person:** Begum Gizem Gokirmak

*Regulatory Affairs and Quality Manager*

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# EC DECLARATION OF CONFORMITY

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| | |
|---|---|
| **Manufacturer** | : Bioeksen R&D Technologies Incorporated Company<br>Resitpasa Mh. Katar Cd. No:4/B-105. Sariyer, Istanbul- TURKEY<br>Web: www.bioeksen.com.tr, e-mail: info@bioeksen.com.tr |
| **Product(s) Name** | : Bio-Speedy® Respiratory Tract RT-qPCR MX-24T Panel |
| **Description** | : Bio-Speedy® Respiratory Tract RT-qPCR MX-24T Panel<br>Ref No: BS-SY-MX24T-25<br>Ref No: BS-SY-MX24T-100 |
| **Classification** | : Other (Neither listed in the Annex II, Nor Self-testing device), GMDN code: 61527 - Multiple-type respiratory pathogen nucleic acid IVD, kit, nucleic acid technique (NAT)<br>Article 9, paragraph 1 of EC Council Directive<br>98/79/EC on In Vitro Medical Diagnostic Devices |
| **Conformity Assessment Route** | : According to Annex III of the IVD Directive 98/79/EC<br>EC declaration of conformity under manufacturer responsibility |
| **Applied Standards** | : All standards stated in the annex on the other page are strictly implemented in our company. |

We hereby declare that the above-mentioned product/s meet the provisions of the EC Council Directive 98/79/EC for in vitro medical diagnostic devices. All supporting documentation is retained under the premises of the manufacturer and manufacturer is exclusively responsible for the declaration of conformity.

**Place of Issue:** Istanbul

***Valid from:*** 16.05.2022

***Signature:***

**Authorized Person:** Begum Gizem Gokirmak

*Regulatory Affairs and Quality Manager*

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*For in vitro diagnostic use only.*
*For laboratory professional use only.*

**Cat No:** BS-DTC-103-100

# Bordetella pertussis, B.parapertussis, B.bronchiseptica and B.holmesii Real-Time PCR Detection Kit

## Package Insert

### 1. Kit Content

**Table 1. Kit Content**

| Component | Intended Use | Amount (10 µL/Rxn) 100 Rxns |
|---|---|---|
| 2X qPCR Mix | Optimized ready-to-use mix for qPCR assay | 1 x 1000 µL |
| Bor 1-Oligo Mix | Specific nucleic acid amplification and detection:<br>FAM: *IS481* gene<br>HEX: Human genome *RNase P* as an internal control | 1 x 250 µL |
| Bor 2-Oligo Mix | Specific nucleic acid amplification and detection:<br>FAM: h*IS1001* gene<br>ROX: *IS1001* gene<br>CY5: *ptxP* gene | 1 x 250 µL |
| NTC | Negative (No Template) Control<br>(Nuclease-Free Water) | 1 x 1000 µL |
| PC-Bor 1 | Positive Control (Synthetic DNA fragment mixture of the targets in the " Bor 1-Oligo Mix") | 1 x 250 µL |
| PC-Bor 2 | Positive Control (Synthetic DNA fragment mixture of the targets in the " Bor 1-Oligo Mix") | 1 x 250 µL |

**Table 2. Storage Requirements and Shelf Life**

| Component | Transport Condition | Storage Condition | Shelf Life |
|---|---|---|---|
| 2X qPCR Mix | -22 °C to +8 °C | -22 °C to -18 °C | 12 months |
| Bor 1-Oligo Mix | | -22 °C to -18 °C | |
| Bor 2-Oligo Mix | | -22 °C to -18 °C | |
| NTC | | -22 °C to -18 °C / +2 °C to +8 °C | |
| PC-Bor 1 | | -22 °C to -18 °C before opening, +2 °C to +8 °C after first thaw | |
| PC-Bor 2 | | -22 °C to -18 °C before opening, +2 °C to +8 °C after first thaw | |

**! Each reagent stored at storage temperature, can be used until the expiration date indicated on the tube following the first opening. The expiration date of the kit is determined by the expiration date of the reagents.**

### 2. Materials Required but Not Provided

**Table 3. Components Required but Not Included with The Test**

| Components Required but Not Included with The Test | |
|---|---|
| **1.** Real-Time PCR instrument with FAM, HEX, ROX, and CY5 channels Ramp rate ≥3 °C/sec | **7.** Reaction tubes and their caps/seals compatible with the qPCR instruments and the reaction volume |
| **2.** Adjustable micropipettes and compatible pipette tips (nuclease-free) | **Extra components recommended to use:** |
| **3.** Centrifuge | **8.** Biosafety cabinet for PCR setup |
| **4.** Vortex | **9.** Cold tube rack (for microcentrifuge tubes and PCR tubes/strips) |
| **5.** Nuclease-free water/viral transport medium/serum physiologic | **10.** PPE (Personal Protective Equipment) |
| **6.** 1.5- or 2-mL microcentrifuge tubes (nuclease-free) | |

### 3. Intended Use and Test Principle

Gram-negative coccobacilli *Bordetella pertussis* cause whooping cough, which is an acute respiratory infection. It is recognized by a paroxysmal cough, but in infants and/or newborns, the symptoms can be fatal. Apart from *B. pertussis*, three other Bordetella subspecies have been linked to human respiratory tract infections: *B. parapertussis*, *B. holmesii*, and *B. bronchiseptica*. These subspecies of Bordetella can develop symptoms similar to those caused by *Bordetella pertussis*, but less severe than *B. pertussis*. Pertussis vaccines do not provide cross-protection against these Bordetella species, which are closely related. Therefore, the detection of Bordetella subspecies in respiratory diseases is necessary in order to make a quick and correct decision on the antibiotic treatment of index patients and their contacts.

***Bio-Speedy® Bordetella pertussis, B.parapertussis, B.bronchiseptica and B.holmesii Real-Time PCR Detection Kit***, is a one-step real-time PCR (qPCR) test intended for the qualitative detection of the DNA from *Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica* and *Bordetella holmesii*. The ***Bio-Speedy® Bordetella pertussis, B.parapertussis, B.bronchiseptica and B.holmesii Real-Time PCR Detection Kit*** is applied to nucleic acids obtained from anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples.

Detection with the kit is achieved via rapid nucleic acid extraction from anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples followed by multiplex qPCR targeting the *IS481, hIS481*, *IS1001* and *IS481* genes for *Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica* and *Bordetella holmesii* in real-time PCR instruments that are equipped with FAM, HEX, ROX, and CY5 detection channels. **The kit allows to achieve qPCR result in less than 30 minutes (Run time may vary depending on the instrument and the thermal cycling protocol)**.

The oligonucleotide set targeting human ***RNase P*** mRNA functions as a control of the sampling, nucleic acid extraction, reverse transcription, and qPCR since the oligonucleotide set targets the exon-exon junction. The kit also contains negative and positive control templates for testing the contamination and the qPCR reagent stability, respectively.

***Bio-Speedy® Bordetella pertussis, B.parapertussis, B.bronchiseptica and B.holmesii Real-Time PCR Detection Kit*** is intended for use by laboratory personnel trained in the techniques of qPCR and *in vitro* diagnostic procedures.

## 4. Analytical Specifications

***Bio-Speedy® Bordetella pertussis, B.parapertussis, B.bronchiseptica and B.holmesii Real-Time PCR Detection Kit*** is validated with ***Bio-Speedy® νNAT® Transfer Tube*** *(Catalog No: BS-NA-513-100),* ***νNAT® Transfer Tube*** *(Catalog No: BS-NA-513m-100),* ***Bio-Speedy® νNAT® Viral Nucleic Acid Buffer*** *(Catalog No: BS-NA-510-100/BS-NA-510-250/BS-NA-510-500/BS-NA-510-1000),* and ***Bio-Speedy® 5min NA*** *(Catalog No: BS-NA-514-100/BS-NA-514-250/BS-NA-514-500/BS-NA-514-1000)* for nucleic acids prepared from anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples.

The qPCR is carried out in 10 µL reaction volume using the ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** Real-Time PCR systems equipped with the **FAM**, **HEX**, **ROX**, and **CY5** detection channel.

For the analysis performed on *Bio-Rad Real-Time PCR systems*, the kit has been validated with white reaction tubes specific to these systems. **The clear reaction tubes result in 5-10 times lower fluorescence signal in the *Bio-Rad* instruments compared to the white tubes.** Besides, device-specific reaction tubes should be used on ***BMS*** instrument (the specified analytical performance of the kit can only be achieved using the validated tubes).

Limit of Detection (LoD) of the ***Bio-Speedy® Bordetella pertussis, B.parapertussis, B.bronchiseptica and B.holmesii Real-Time PCR Detection Kit*** is determined as 150-200 cp/mL for *Bordetella pertussis, B.parapertussis, B.bronchiseptica ve B.holmesii*.

The exclusivity of the kit was tested on different pathogens. No cross-reaction was observed in analytical specificity studies performed on reference strains and field isolates. The sensitivity and specificity of the kit were determined as 100.00% and 100.00%, respectively.

## 5. Collection, Storage and Shipment of Clinical Specimens

Clinical samples (anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples) are collected from individuals by a healthcare provider in accordance with the specimen collection guidelines. Anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, and oral/saliva swab samples are transferred into the ***Bio-Speedy® νNAT® Transfer Tube*** *(Catalog No: BS-NA-513-100)* or ***νNAT® Transfer Tube*** *(Catalog No: BS-NA-513m-100)* containing 2 mL of the ***νNAT® reagent*** or into a sterile transport tube containing 3 mL of Viral Transport Medium (VTM) (Preparation of Viral Transport Medium, Centers for Disease Control and Prevention, SOP#: DSR-052-05). Bronchoalveolar lavage and nasopharyngeal aspirate samples should be transferred into sterile containers containing 3 mL of VTM. Other sample types (saliva, gargle, and sputum samples) should be transferred into preservative-free sterile tubes.

Specimens should be sent to the laboratory within 4 hours after collection at 2 °C to 8 °C. If a delay in delivery for more than 3 days is expected, the samples should be frozen at -70 °C and delivered with dry ice. It is important that the samples should not be exposed to the repeated freeze-thaw in order to prevent Nucleic Acid degredation.

Specimens in the VTM can be stored at 2 °C to 8 °C for up to 72 hours and specimens in the ***νNAT®Transfer Tube*** or ***Bio-Speedy® νNAT® Transfer Tube*** can be stored at 2 °C to 8 °C for up to 3 months. If a delay in the qPCR test is expected, specimens can be stored at -70 °C. If not available, specimens can be stored at -20 °C.

## 6. Preparation of Nucleic Acid Samples

One minute after the collection, the swab samples in the ***Bio-Speedy® νNAT®Transfer Tube*** or ***νNAT® Transfer Tube*** can directly be used in qPCR.

The dry swab samples are combined with 1 mL of "molecular grade water: ***Bio-Speedy® νNAT® Viral Nucleic Acid Buffer (10X concentrated)*** (in a 9:1 ratio)" mixture. The mixture containing the swab can directly be used in qPCR after 1 minute of incubation at room temperature.

The samples in the VTM or the saline are combined with the ***Bio-Speedy® νNAT® Viral Nucleic Acid Buffer (10X concentrated)*** in a 9:1 (sample: ***νNAT®***) ratio. The mixture can directly be used in qPCR after 1 minute of incubation at room temperature.

***Bio-Speedy® 5min NA*** is used according to the manufacturer's instruction.

The samples are combined with the ***5min NA reagent*** in a 19:1 (Sample:***5min NA***) ratio (e.g., 47.5 µL Sample:2.5 µL ***5min NA***). The mixture is incubated in a thermal cycler for 2 min at 65 °C, 3 min at 95 °C, and 1 sec at 40 °C. Afterward, the samples are ready to use in the qPCR reaction.

## 7. Warnings

1. Specimen processing should be performed in accordance with national biological safety recommendations.
2. Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
3. All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate. Sampling should be carried out by personnel with sufficient knowledge and experience.
4. The kit should be stored away from nucleic acid sources and PCR amplicons.
5. Except for fluid transfers, nucleic acid and positive control tubes should always be kept closed.
6. To prevent contamination of the reaction mixture by previously amplified target sequences, maintain separate work areas, dedicated equipment.
7. Different sets of laboratory coats should be worn pre- and post-PCR.
8. The micropipettes used for pipetting PCR mixes and template nucleic acids should be separate. Filtered and nuclease-free tips should be used.
9. Cotton or calcium alginate swabs or swabs with wooden sticks should not be used since they may contain substances that inactivate some pathogens and inhibit PCR.
10. It is recommended to use swabs with breakable shaft to prevent contamination during sampling.
11. The components in the kit should not be mixed with different lot numbers or chemicals of the same name but from different manufacturers.
12. Master stock reagents should be kept on the cold block during the PCR setup.
13. Kit components should be mixed by gently shaking before use.
14. Maintenance/calibration interval should be determined for all instruments and equipment used with the kit.
15. To avoid false positives due to amplified material, the PCR completed reaction tubes should be disposed of before opening in the laboratory.
16. The wipeable surfaces of the rooms, benches, and devices should be cleaned regularly with freshly diluted 10% bleach solution (0.5% NaClO).
17. Dispose of waste in a designated matter in accordance with local, regional, and federal regulations.

## 8. qPCR Application Protocol

**Table 4. Reaction Set-up and qPCR Program Details**

| Reaction Setup | | qPCR Program | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fast qPCR Protocol | | | | Touchdown qPCR Protocol | | | |
| | | *CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)* and *Mic qPCR (Bio Molecular System - BMS)* | | | | *CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)* and *Mic qPCR (Bio Molecular System - BMS)* | | | |
| **Reagent** | **Volume per Rxn** | **Step** | **Cycle No.** | **Temperature** | **Duration** | **Step** | **Cycle No.** | **Temperature** | **Duration** |
| 2X qPCR Mix | 5 µL | **Reverse Transcription** | 1 Cycle | 52 °C | 3 min | **Reverse Transcription** | 1 Cycle | 52 °C | 3 min |
| | | **Pre-incubation** | 1 Cycle | 95 °C | 10 sec | **Pre-incubation** | 1 Cycle | 95 °C | 10 sec |
| Oligo Mix | 2.5 µL | **Denaturation** | 5 Cycles | 95 °C | 1 sec | **Denaturation** | 12 Touchdown Cycles: 1 °C decrement in annealing temperature per cycle | 95 °C | 1 sec |
| | | **Annealing/Extension** | | 55 °C | 12 sec | **Annealing/Extension** | | 67 °C – 56 °C | 10 sec |
| Template Nucleic Acid | 2.5 µL | **Denaturation** | 35 Cycles | 85 °C | 1 sec | **Denaturation** | 35 Cycles | 85 °C | 1 sec |
| | | **Annealing/Extension** | | 55 °C | 1 sec | **Annealing/Extension** | | 55 °C | 10 sec |
| **TOTAL REACTION VOLUME** | **10 µL** | **Detection (Reading)** | | (FAM-Green)/(HEX-Yellow)/(ROX-Orange)/(CY5-Red) | | **Detection (Reading)** | | (FAM-Green)/(HEX-Yellow)/(ROX-Orange)/(CY5-Red) | |

**WARNING: The qPCR program template of the instrument's software must be downloaded from the manufacturer's website to avoid errors in the manual entry.**

**QR Codes of the thermal profiles for Bio-Rad and Mic.**

https://www.bioeksen.com.tr/files/bordetella_pertussis_parapertussis_bronchiseptica_holmesii/

## 9. Interpretation of the Assay Results

**For the Fast qPCR Protocol:**

- The threshold level should be set to 200 RFU for ***CFX96 Touch™/CFX96™ Dx (Bio-Rad)*** **and** ***CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments to calculate Cq values. All other default analysis options in the related software should not be changed for ***CFX96 Touch™/CFX96™ Dx (Bio-Rad)*** **and** ***CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments. For ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)***, "**Non-Assay Green/Parameters/Dynamic**" and "**Auto-Threshold**" options should be selected to calculate Cq values.
- Shape of the amplification curves obtained in the **FAM**/**HEX**/**ROX**/**CY5** channels should be examined for all reaction wells returning with Cq values. Cq values should be used in the further interpretation steps if their amplification curve shapes are sigmoidal. **Non-sigmoidal curves should be recorded as "negative"**. The result is recorded as "**positive**" if **Cq≤33**.
- **For samples with a suspected sigmoidal curve pattern under the threshold in the FAM/ROX/CY5 channels**, Cq-HEX (IC) should be examined. If the Cq-HEX≤30, the sample is reported as negative. If the Cq-HEX>30, the test should be repeated after freezing and thawing the sample. If the problem continues after the freezing and thawing, a new sample is requested.

**For the Touchdown qPCR Protocol:**

- All default analysis options (e.g. **auto-calculated threshold**) in the related software of ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System – BMS)*** instruments should not be changed to calculate Cq values.
- Shape of the amplification curves obtained in the **FAM**/**HEX**/**ROX**/**CY5** channels should be examined for all reaction wells returning with Cq values. All the **sigmoidal curves above the threshold** should be recorded as "**positive**" and their Cq values should be recorded. **Non-sigmoidal curves should be recorded as "negative"**.
- For **samples with a sigmoidal curve below the auto-calculated threshold** for the ***CFX96 Touch™/CFX96™ Dx (Bio-Rad) and CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments, **the threshold level should be manually set to 200 RFU. If the sigmoidal curve exceeds the threshold, the Cq value should be recorded as "35"** and the sample should be reported as "**positive**".
- For **samples with a sigmoidal curve below the auto-calculated threshold** for ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** should be reported as "**positive**" and **their Cq values should be recorded as "35"**.

**Table 5. Expected Performance of the Kit Controls**

| Control Type | Control Name | Purpose | Expected Results and Cq Values | |
|---|---|---|---|---|
| | | | ***Internal Control*** **(HEX)** | ***Target*** **(FAM, ROX, CY5)** |
| **Negative Control** | NTC | Contamination control during qPCR | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **No template addition** | NRC | Reagent contamination control | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **Positive Control** | PC | Reagent integrity | **Detected** (Cq≤33) | **Detected** (Cq≤33) |
| **Internal/Extraction Control** | IC | To monitor the integrity of nucleic acid extraction and qPCR from each sample | **Detected** (Cq≤33)<br>If IC Cq>33 check the target Cq | For the Touchdown qPCR Protocol;<br>If target Cq≤35.0, conclude it as IC is valid<br>For the Fast qPCR Protocol;<br>If target Cq≤33.0, conclude it as IC is valid |

If any control does not perform as described above, the run is considered invalid, and the test is repeated.

1. **Invalid PC (Cq>33 in any channel):** It is recommended to contact the manufacturer, renew the reagents, and repeat the reaction.

2. **Invalid NTC (No Cq in any channel):** Repeat the analysis by paying attention to the "**Warnings**" section.
3. **Invalid NRC (No Cq in any channel):** Contact the manufacturer, renew the reagents, and repeat the reaction.
4. **Invalid IC (Cq>33 in HEX channel and no Cq in the other channels):** Repeat the analysis. If the problem continues, then conclude it as an invalid PCR template.

If all the controls are valid, proceed to the interpretation of the results.

**Table 6. Interpretation of Patient Samples**

| Target | Internal Control | Results Interpretation | Action |
|---|---|---|---|
| Positive (+) | Positive (+) | Results are VALID, Target DNA is detected | Report it as **POSITIVE** |
| Positive (+) | Negative (-) | Results are VALID, Target DNA is detected | Report it as **POSITIVE** |
| Negative (-) | Positive (+) | Results are VALID, Target DNA is not detected | Report it as **NEGATIVE** |
| Negative (-) | Negative (-) | Results are INVALID (sampling/extraction/inhibition problem) | Re-extract the specimen and perform testing again. If the result is still invalid, a new specimen should be obtained. If an additional clinical sample is unavailable, report it as **INVALID** |

| Target | Results Interpretation | Expected Results and Cq values | Action |
|---|---|---|---|
| ***Bordetella pertussis*** | *IS481* and *ptxP* should be positive | **Cq values: Independent** | Report as *Bordetella pertussis* **POSITIVE** |
| ***Bordetella parapertussis*** | *IS1001* should be positive | **Cq values: *IS1001<IS481*** | Report as *Bordetella parapertussis* **POSITIVE** |
| ***Bordetella holmesii*** | *IS481* and *hIS1001* should be positive | **Cq values: Independent** | Report as *Bordetella holmesii* **POSITIVE** |
| ***Bordetella bronchiseptica*** | *IS1001* and *IS481* should be positive | **Cq values: IS481<IS1001** | Report as *Bordetella bronchiseptica* **POSITIVE** |

**WARNING: On the web page linked with the QR code, examples of the sigmoidal amplification curves are given. The results obtained with this kit should NOT be interpreted without examining these samples.**

## 10. Limitations

- ***Bio-Speedy® Bordetella pertussis, B.parapertussis, B.bronchiseptica and B.holmesii Real-Time PCR Detection Kit*** is intended for use by laboratory personnel trained in the techniques of qPCR and *in vitro* diagnostic procedures.
- The clinical specimens shall be collected by a healthcare provider in accordance with the specimen collection guidelines.
- A false-negative result may occur if a specimen is improperly collected, transported, or handled.
- The use of cotton or calcium alginate swabs or swabs with wooden sticks can lead to false-negative results since they may contain substances that inactivate some pathogens and inhibit PCR.
- Mutations within the target regions of the ***Bio-Speedy® Bordetella pertussis, B.parapertussis, B.bronchiseptica and B.holmesii Real-Time PCR Detection Kit*** could affect primer and/or probe binding resulting in failure to detect the presence of agents.
- Inhibitors or other types of interference may produce a false-negative result. False-negative results may also occur if inadequate numbers of organisms are present in the specimen.

## 11. Explanation of Symbol

| Symbol | Title of Symbol | Symbol | Title of Symbol | Symbol | Title of Symbol |
|---|---|---|---|---|---|
| CE | European Conformity CE Mark | LOT | Batch code | Σ | Contains sufficient for <*n*> tests |
| IVD | *In vitro* diagnostic *medical device* | REF | Catalogue number | | Keep away from sunlight |
| | Manufacturer | NON STERILE | Non-*sterile* | | Protect from heat and radioactive sources |
| | Use-by date | | Consult *instructions for use* or consult electronic *instructions for use* | | Do not use if package is damaged and consult *instructions for use* |
| CONTROL - | Negative control | | Caution | | Keep dry |
| CONTROL + | Positive control | | Temperature limit | | Keep it upright |
| CONTROL | Control | | | | |

## 12. Manufacturer and Technical Support

**Bioeksen R&D Technologies Incorporated Company**
**Address:** Resitpasa Mh. Katar Cd., 4/B-105. 34467, Sariyer, Istanbul, TURKEY.
**Phone:** +90 (212) 285 10 17, **Fax:** +90 (212) 285 10 18
**Web**: www.bioeksen.com.tr, **E-mail:** info@bioeksen.com.tr,
**Technical Support**: support@bioeksen.com.tr

**Notice to User:** Please inform us about product-related incidents at "**vigilance@bioeksen.com.tr**" within 24 hours.

*For in vitro diagnostic use only.*
*For laboratory professional use only.*

**Cat No:** BS-BNV-DTC-322-100

# West Nile Virus Real Time PCR Detection Kit

## Package Insert

### 1. Kit Content

**Table 1. Kit Content**

| Component | Intended Use | Amount (10 µL/Rxn) 100 Rxns |
|---|---|---|
| 2X Prime Script Mix | Optimized ready-to-use mix for RT-qPCR assay | 1 x 500 µL |
| WNV Oligo Mix | Specific nucleic acid amplification and detection:<br>FAM: West Nile virus-specific *E* gene<br>HEX: Human genome *RNase P* as an internal control | 1 x 250 µL |
| NTC | Negative (No Template) Control<br>(Nuclease-Free Water) | 1 x 1000 µL |
| PC-WNV | Positive Control (Synthetic RNA fragment mixture of the targets in the "WNV Oligo Mix") | 1 x 250 µL |

**Table 2. Storage Requirements and Shelf Life**

| Component | Transport Condition | Storage Condition | Shelf Life |
|---|---|---|---|
| 2X Prime Script Mix | -22 °C to +8 °C | -22 °C to -18 °C | 12 months |
| WNV Oligo Mix | | -22 °C to -18 °C | |
| NTC | | -22 °C to -18 °C / +2 °C to +8 °C | |
| PC-WNV | | -22 °C to -18 °C before opening, +2 °C to +8 °C after the first thaw | |

**! Each reagent stored at storage temperature can be used until the expiration date indicated on the tube following the first opening. The expiration date of the kit is determined by the expiration date of the reagents.**

### 2. Materials Required but Not Provided

**Table 3. Components Required but Not Included with The Test**

| Components Required but Not Included with The Test | |
|---|---|
| **1.** Real-Time PCR instrument with FAM and HEX channels Ramp rate ≥3 °C/sec | **7.** Reaction tubes and their caps/seals compatible with the qPCR instruments and the reaction volume |
| **2.** Adjustable micropipettes and compatible pipette tips (nuclease-free) | **Extra components recommended to use:** |
| **3.** Centrifuge | **8.** Biosafety cabinet for PCR setup |
| **4.** Vortex | **9.** Cold tube rack (for microcentrifuge tubes and PCR tubes/strips) |
| **5.** Nuclease-free water/viral transport medium/serum physiologic | **10.** PPE (Personal Protective Equipment) |
| **6.** 1.5- or 2-mL microcentrifuge tubes (nuclease-free) | |

### 3. Intended Use and Test Principle

The West Nile virus (WNV) is a single-stranded RNA flavivirus spread by mosquitoes that predominantly affects birds but can also infect humans and horses infrequently. WNV had only been identified in the Eastern hemisphere up until the viral infection was discovered in birds in New York City in 1999, with a wide spread in Africa, Asia, the Middle East, and Europe. The majority of WNV carriers do not exhibit any symptoms. West Nile fever, which has moderate symptoms including headache, myalgia, and occasionally a skin rash on the trunk of the body, is thought to affect 20% of individuals who contract the virus. Meningitis or encephalitis occurs in around 1 out of 150 WNV infections (less than 1%). Hospitalized patients during recent outbreaks suffered case fatality rates that varied from 4% to 14%. The most significant risk factor for mortality is advanced age, and patients over the age of 70 are at very high risk.

***Bio-Speedy® West Nile Virus Real Time PCR Detection Kit*** is a one-step reverse transcription and real-time PCR (RT-qPCR) test intended for the qualitative detection of the RNA from West Nile virus (WNV). The ***Bio-Speedy® West Nile Virus Real Time PCR Detection Kit*** is applied to nucleic acids obtained from serum, plasma, and whole blood samples. The kit is non-automated and functions as an aid to diagnosis.

Detection with the kit is achieved via rapid nucleic acid extraction from serum, plasma, and whole blood samples followed by multiplex RT-qPCR targeting the West Nile virus (WNV) specific *Envelope (E) gene* in real-time PCR instruments that are equipped with FAM and HEX detection channels. **The kit allows to achieve RT-qPCR results in 43 minutes (Run time may vary depending on the instrument)**.

The oligonucleotide set targeting human ***RNase P*** mRNA functions as a control of the sampling, nucleic acid extraction, reverse transcription, and qPCR since the oligonucleotide set targets the exon-exon junction. The kit also contains negative and positive control templates for testing the contamination and the RT-qPCR reagent stability, respectively.

***Bio-Speedy® West Nile Virus Real Time PCR Detection Kit*** is intended for use by laboratory personnel trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures.

### 4. Analytical Specifications

***Bio-Speedy® West Nile Virus Real Time PCR Detection Kit*** is validated with a robotic extraction system such as ***Zybio EXM3000 Nucleic Acid Isolation System*** *(Model No: EXM3000)* for nucleic acids prepared from serum, plasma, and whole blood samples.

The RT-qPCR is carried out in 10 µL reaction volume using the ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** Real-Time PCR systems equipped with the FAM and HEX detection channels.

For the analysis performed on *Bio-Rad Real-Time PCR systems*, the kit has been validated with white reaction tubes specific to these systems. **The clear reaction tubes result in 5-10 times lower fluorescence signal in the *Bio-Rad* instruments compared to the white tubes.** Besides, device-specific reaction tubes should be used on ***BMS*** instruments (the specified analytical performance of the kit can only be achieved using the validated tubes).

Limit of Detection (LoD) of the ***Bio-Speedy® West Nile Virus Real Time PCR Detection Kit*** is determined as 250 cp/mL for the West Nile virus (WNV) for serum, plasma, and whole blood samples extracted using the ***Zybio EXM3000 Nucleic Acid Isolation System***.

The exclusivity of the kit was tested on different pathogens. No cross-reaction was observed in analytical specificity studies performed on reference strains and field isolates. The sensitivity and specificity of the kit were determined as 100.00% and 100.00%, respectively.

## 5. Collection, Storage, and Shipment of Clinical Specimens

Collect whole blood into commercially available anticoagulant-treated tubes, e.g., EDTA-treated (lavender tops) or citrate-treated (light blue tops) for whole blood samples. Whole blood samples in tubes are preferably stored at 2 °C to 8 °C and transferred to the laboratory within 24 hours at the latest. For long-term storage, samples should be stored at -20°C.

Following the centrifugation of serum or plasma samples, it is crucial to immediately transfer the serum or plasma samples using a Pasteur pipette into a sterile polypropylene tube. During handling, the samples should be kept between 2 and 8 °C. The serum or plasma samples should be portioned into 0.5 ml aliquots, stored, and transported at -20°C or lower if they won't be analyzed immediately. It is important to avoid freeze-thaw cycles.

## 6. Preparation of Nucleic Acid Samples

The automated ***Zybio EXM3000 Nucleic Acid Isolation System*** extraction is used according to the manufacturer's instructions.

## 7. Warnings

1. Specimen processing should be performed in accordance with national biological safety recommendations.
2. Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
3. All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate. Sampling should be carried out by personnel with sufficient knowledge and experience.
4. The kit should be stored away from nucleic acid sources and PCR amplicons.
5. Except for fluid transfers, nucleic acid, and positive control tubes should always be kept closed.
6. To prevent contamination of the reaction mixture by previously amplified target sequences, maintain separate work areas, and dedicated equipment.
7. Different sets of laboratory coats should be worn pre- and post-PCR.
8. The micropipettes used for pipetting PCR mixes and template nucleic acids should be separate. Filtered and nuclease-free tips should be used.
9. The components in the kit should not be mixed with different lot numbers or chemicals of the same name but from different manufacturers.
10. Master stock reagents should be kept on the cold block during the PCR setup.
11. Kit components should be mixed by gently shaking before use.
12. Maintenance/calibration interval should be determined for all instruments and equipment used with the kit.
13. To avoid false positives due to amplified material, the PCR-completed reaction tubes should be disposed of before opening in the laboratory.
14. The wipeable surfaces of the rooms, benches and devices should be cleaned regularly with freshly diluted 10% bleach solution (0.5% NaClO).
15. Dispose of waste in a designated matter in accordance with local, regional, and federal regulations.

## 8. RT-qPCR Application Protocol

**Table 4. Reaction Set-up and RT-qPCR Program Details**

<table>
<tr><th colspan="2" rowspan="2">Reaction Setup</th><th colspan="4">RT-qPCR Program</th><th>QR Code for Thermal Protocol</th></tr>
<tr><th colspan="4">CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad) and Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)</th><td rowspan="10">https://www.bioeksen.com.tr/files/wnv</td></tr>
<tr><th>Reagent</th><th>Volume/Rxn</th><th>Step</th><th>Cycle No.</th><th>Temperature</th><th>Duration</th></tr>
<tr><td rowspan="2">2X Prime Script Mix</td><td rowspan="2">5 µL</td><td>Reverse Transcription</td><td>1 Cycle</td><td>52 °C</td><td>3 min</td></tr>
<tr><td>Pre-Incubation</td><td>1 Cycle</td><td>95 °C</td><td>10 sec</td></tr>
<tr><td rowspan="2">WNV Oligo Mix</td><td rowspan="2">2.5 µL</td><td>Denaturation</td><td rowspan="2">12 Touch Down Cycles:<br>1 °C decrement in annealing temperature per cycle</td><td>95 °C</td><td>1 sec</td></tr>
<tr><td>Annealing and Extension</td><td>67 °C – 56 °C</td><td>10 sec</td></tr>
<tr><td rowspan="2">Template Nucleic Acid</td><td rowspan="2">2.5 µL</td><td>Denaturation</td><td rowspan="3">35 Cycles</td><td>85 °C</td><td>1 sec</td></tr>
<tr><td>Annealing and Extension</td><td>55 °C</td><td>10 sec</td></tr>
<tr><td>Total Reaction Volume</td><td>10 µL</td><td>Detection (Reading)</td><td colspan="2">(FAM-Green)/(HEX-Yellow)</td></tr>
</table>

**WARNING: The RT-qPCR program template of the instrument's software must be downloaded from the manufacturer's website to avoid errors in the manual entry.**

## 9. Interpretation of the Assay Results

- All default analysis options (e.g. **auto-calculated threshold**) in the related software of ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System – BMS)*** instruments should not be changed to calculate Cq values.
- Shape of the amplification curves obtained in the **FAM**/**HEX** channels should be examined for all reaction wells returning with Cq values. All the **sigmoidal curves above the threshold** should be recorded as "**positive,**" and their Cq values should be recorded. **Non-sigmoidal curves** should be recorded as "**negative**".

- For samples with a sigmoidal curve below the auto-calculated threshold for the ***CFX96 Touch™/CFX96™ Dx (Bio-Rad) and CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments, **the threshold level should be manually set to 200 RFU. If the sigmoidal curve exceeds the threshold, the Cq value should be recorded as "35,"** and the sample should be reported as "**positive**".

- For **samples with a sigmoidal curve below the auto-calculated threshold** for ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** should be reported as "**positive,**" and **their Cq values should be recorded as "35"**.

**Table 5. Expected Performance of the Kit Controls**

| Control Type | Control Name | Purpose | Expected Results and Cq Values | |
|---|---|---|---|---|
| | | | *Internal Control* (HEX) | *Target* (FAM) |
| **Negative Control** | NTC | Contamination control during RT-qPCR | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **No template addition** | NRC | Reagent contamination control | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **Positive Control** | PC | Reagent integrity | **Detected** (Cq≤33) | **Detected** (Cq≤33) |
| **Internal/Extraction Control** | IC | To monitor the integrity of nucleic acid extraction and RT-qPCR from each sample | **Detected** (Cq≤33)<br>If IC Cq>33 check the target Cq | If target Cq≤35.0, conclude it as IC is valid |

If any control does not perform as described above, the run is considered invalid, and the test is repeated.

1. **Invalid PC (Cq>33 in any channel):** It is recommended to contact the manufacturer, renew the reagents, and repeat the reaction.
2. **Invalid NTC (No Cq in any channel):** Repeat the analysis by paying attention to the "**Warnings**" section.
3. **Invalid NRC (No Cq in any channel):** Contact the manufacturer, renew the reagents, and repeat the reaction.
4. **Invalid IC (Cq>33 in HEX channel and no Cq in the other channels):** Repeat the analysis. If the problem continues, then conclude it as an invalid PCR template.

If all the controls are valid, proceed to the interpretation of the results.

**Table 6. Interpretation of Patient Samples**

| Target | Internal Control | Results Interpretation | Action |
|---|---|---|---|
| **Positive (+)** | **Positive (+)** | Results are VALID,<br>Target RNA is detected | Report it as **POSITIVE** |
| **Positive (+)** | **Negative (-)** | Results are VALID,<br>Target RNA is detected | Report it as **POSITIVE** |
| **Negative (-)** | **Positive (+)** | Results are VALID,<br>Target RNA is not detected | Report it as **NEGATIVE** |
| **Negative (-)** | **Negative (-)** | Results are INVALID<br>(sampling/extraction/inhibition problem) | Re-extract the specimen and perform testing again. If the result is still invalid, a new specimen should be obtained. If an additional clinical sample is unavailable, report it as **INVALID** |

**WARNING: On the web page linked with the QR code, examples of the sigmoidal amplification curves are given. The results obtained with this kit should NOT be interpreted without examining these samples.**

## 10. Limitations

- ***Bio-Speedy® West Nile Virus Real Time PCR Detection Kit*** is intended for use by laboratory personnel trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures.
- The clinical specimens shall be collected by a healthcare provider in accordance with the specimen collection guidelines.
- A false-negative result may occur if a specimen is improperly collected, transported, or handled.
- Mutations within the target regions of the ***Bio-Speedy® West Nile Virus Real Time PCR Detection Kit*** could affect primer and/or probe binding resulting in failure to detect the presence of the virus.
- Inhibitors or other types of interference may produce a false-negative result. False-negative results may also occur if inadequate numbers of organisms are present in the specimen.

## 11. Explanation of Symbol

| Symbol | Title of Symbol | Symbol | Title of Symbol | Symbol | Title of Symbol |
|---|---|---|---|---|---|
| CE | European Conformity CE Mark | LOT | Batch code | Σ | Contains sufficient for <*n*> tests |
| IVD | *In vitro* diagnostic *medical device* | REF | Catalogue number | | Keep away from sunlight |
| | Manufacturer | NON STERILE | Non-*sterile* | | Protect from heat and radioactive sources |
| | Use-by date | | Consult *instructions for use* or consult electronic *instructions for use* | | Do not use if package is damaged and consult *instructions for use* |
| CONTROL - | Negative control | | Caution | | Keep dry |
| CONTROL + | Positive control | | Temperature limit | | Keep it upright |
| CONTROL | Control | | | | |

## 12. Manufacturer and Technical Support

**Bioeksen R&D Technologies Incorporated Company**
**Address:** Resitpasa Mh. Katar Cd., 4/B-105. 34467, Sariyer, Istanbul, TURKEY.
**Phone:** +90 (212) 285 10 17, **Fax:** +90 (212) 285 10 18
**Web**: www.bioeksen.com.tr, E-**mail:** info@bioeksen.com.tr,
**Technical Support**: support@bioeksen.com.tr

**Notice to User:** Please inform us about product-related incidents at "**vigilance@bioeksen.com.tr**" within 24 hours.

*For in vitro diagnostic use only.*
*For laboratory professional use only.*

**Cat No:** BS-DTC-V-224-100/BS-DTC-V-224-100

# Bacillus anthracis Real-Time PCR Detection Kit

## Package Insert

### 1. Kit Content

**Table 1. Kit Content**

| Component | Intended Use | Amount (10 µL/Rxn) | |
|---|---|---|---|
| | | 25 Rxns | 100 Rxns |
| 2X qPCR Mix | Optimized ready-to-use mix for qPCR assay | 1 x 125 µL | 1 x 500 µL |
| BA Oligo Mix | Specific nucleic acid amplification and detection:<br>FAM: *Bacillus anthracis* specific *lef* and *capA* genes<br>HEX: Human genome *RNase P* as an internal control | 1 x 62.5 µL | 1 x 250 µL |
| NTC | Negative (No Template) Control<br>(Nuclease-Free Water) | 1 x 1000 µL | 1 x 1000 µL |
| PC-BA | Positive Control (Synthetic DNA fragment mixture of the targets in the "BA Oligo Mix") | 1 x 100 µL | 1 x 250 µL |

**Table 2. Storage Requirements and Shelf Life**

| Component | Transport Condition | Storage Condition | Shelf Life |
|---|---|---|---|
| 2X qPCR Mix | -22 °C to +8 °C | -22 °C to -18 °C | 12 months |
| BA Oligo Mix | | -22 °C to -18 °C | |
| NTC | | -22 °C to -18 °C / +2 °C to +8 °C | |
| PC-BA | | -22 °C to -18 °C before opening, +2 °C to +8 °C after first thaw | |

**! Each reagent stored at storage temperature can be used until the expiration date indicated on the tube following the first opening. The expiration date of the kit is determined by the expiration date of the reagents.**

### 2. Materials Required but Not Provided

**Table 3. Components Required but Not Included with The Test**

| Components Required but Not Included with The Test | |
|---|---|
| **1.** Real-Time PCR instrument with FAM and HEX channels Ramp rate ≥3 °C/sec | **7.** Reaction tubes and their caps/seals compatible with the qPCR instruments and the reaction volume |
| **2.** Adjustable micropipettes and compatible pipette tips (nuclease-free) | **Extra components recommended to use:** |
| **3.** Centrifuge | **8.** Biosafety cabinet for PCR setup |
| **4.** Vortex | **9.** Cold tube rack (for microcentrifuge tubes and PCR tubes/strips) |
| **5.** Nuclease-free water/viral transport medium/serum physiologic | **10.** PPE (Personal Protective Equipment) |
| **6.** 1.5- or 2-mL microcentrifuge tubes (nuclease-free) | |

### 3. Intended Use and Test Principle

The anthrax-causing bacterium, *Bacillus anthracis* (*B. anthracis*), is a Gram-positive spore-forming bacillus that is commonly found in the soil in endemic regions. Anthrax is a zoonotic illness that is mostly spread by domesticated animals and herbivores. Less often, anthrax infects humans. Humans may contract these bacteria through the skin, gastrointestinal tract, or respiratory system. Fever, dysphagia, respiratory discomfort, regional lymphadenopathy, and significant neck swelling are clinical manifestations. Initial symptoms are usually nonspecific, delaying diagnosis. Later symptoms include fever, anorexia, vomiting, severe abdominal pain, haematemesis, severe bloody diarrhea, and developing ascites. Anthrax has a mortality rate of 25%–60%, and it could possibly be 100% if the diagnosis is delayed.

***Bio-Speedy® Bacillus anthracis Real-Time PCR Detection Kit*** is a one-step real-time PCR (qPCR) test intended for the qualitative detection of the DNA from *Bacillus anthracis*. The ***Bio-Speedy® Bacillus anthracis Real-Time PCR Detection Kit*** is applied to nucleic acids obtained from Whole blood, serum, and cerebral spinal fluid (CSF) samples. The kit is non-automated and functions as an aid to diagnosis.

Detection with the kit is achieved via rapid nucleic acid extraction from Whole blood, serum, and cerebral spinal fluid (CSF) samples followed by multiplex qPCR targeting the *Bacillus anthracis* specific *lethal factor* ***(lef)*** and Capsule biosynthesis protein (***capA***) genes in real-time PCR instruments that are equipped with FAM and HEX detection channels. **The kit allows to achieve qPCR results in 43 minutes (Run time may vary depending on the instrument)**.

The oligonucleotide set targeting human ***RNase P*** mRNA functions as a control of the sampling, nucleic acid extraction, and qPCR since the oligonucleotide set targets the exon-exon junction. The kit also contains negative and positive control templates for testing the contamination and the qPCR reagent stability, respectively.

***Bio-Speedy® Bacillus anthracis Real-Time PCR Detection Kit*** is intended for use by laboratory personnel trained in the techniques of qPCR and *in vitro* diagnostic procedures.

### 4. Analytical Specifications

***Bio-Speedy® Bacillus anthracis Real-Time PCR Detection Kit*** is validated with robotic extraction system such as ***Zybio EXM3000 Nucleic Acid Isolation System*** *(Model No: EXM3000)* for nucleic acids prepared from Whole blood, serum, and cerebral spinal fluid (CSF) samples.

The qPCR is carried out in 10 µL reaction volume using the ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** Real-Time PCR systems equipped with the FAM and HEX detection channels.

For the analysis performed on *Bio-Rad Real-Time PCR systems*, the kit has been validated with white reaction tubes specific to these systems. **The clear reaction tubes result in 5-10 times lower fluorescence signal in the *Bio-Rad* instruments compared to the white tubes.** Besides, device-specific reaction tubes should be used on ***BMS*** instrument (the specified analytical performance of the kit can only be achieved using the validated tubes).

Limit of Detection (LoD) of the ***Bio-Speedy® Bacillus anthracis Real-Time PCR Detection Kit*** is determined as 150 CFU/mL for *Bacillus anthracis*.

The exclusivity of the kit was tested on different pathogens. No cross-reaction was observed in analytical specificity studies performed on reference strains and field isolates. The sensitivity and specificity of the kit were determined as 100.00% and 100.00%, respectively.

## 5. Collection, Storage, and Shipment of Clinical Specimens

Collect whole blood into commercially available anticoagulant-treated tubes e.g. EDTA-treated (lavender tops) or citrate-treated (light blue tops) for whole blood samples. Whole blood samples in tubes are preferably stored at 2 °C to 8 °C and transferred to the laboratory within 24 hours at the latest. For long-term storage, samples should be stored at -20°C.

Following the centrifugation of serum samples, it is crucial to immediately transfer the serum samples using a Pasteur pipette into a sterile polypropylene tube. During handling, the samples should be kept between 2 and 8 °C. The serum samples should be portioned into 0.5 ml aliquots, stored, and transported at -20°C or lower if they won't be analyzed immediately. It is important to avoid freeze-thaw cycles.

CSF samples should be collected by a healthcare provider in accordance with the specimen collection guidelines. CSF samples are transferred to the laboratory in a sterile transport tube. The samples should be transported to the laboratory within 2 days at 2-8°C. If a delay in shipment is expected, samples should be frozen at -70°C and shipped with dry ice. It is important that samples should not be exposed to repeated freeze-thaw.

## 6. Preparation of Nucleic Acid Samples

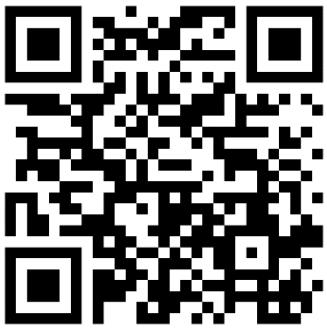
The automated ***Zybio EXM3000 Nucleic Acid Isolation System*** extraction is used according to the manufacturer's instructions.

## 7. Warnings

1. Specimen processing should be performed in accordance with national biological safety recommendations.
2. Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
3. All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate. Sampling should be carried out by personnel with sufficient knowledge and experience.
4. The kit should be stored away from nucleic acid sources and PCR amplicons.
5. Except for fluid transfers, nucleic acid, and positive control tubes should always be kept closed.
6. To prevent contamination of the reaction mixture by previously amplified target sequences, maintain separate work areas, and dedicated equipment.
7. Different sets of laboratory coats should be worn pre- and post-PCR.
8. The micropipettes used for pipetting PCR mixes and template nucleic acids should be separate. Filtered and nuclease-free tips should be used.
9. The components in the kit should not be mixed with different lot numbers or chemicals of the same name but from different manufacturers.
10. Master stock reagents should be kept on the cold block during the PCR setup.
11. Kit components should be mixed by gently shaking before use.
12. Maintenance/calibration interval should be determined for all instruments and equipment used with the kit.
13. To avoid false positives due to amplified material, the PCR-completed reaction tubes should be disposed of before opening in the laboratory.
14. The wipeable surfaces of the rooms, benches and devices should be cleaned regularly with freshly diluted 10% bleach solution (0.5% NaClO).
15. Dispose of waste in a designated matter in accordance with local, regional, and federal regulations.

## 8. qPCR Application Protocol

**Table 4. Reaction Set-up and qPCR Program Details**

<table>
<tr><th colspan="2" rowspan="2">Reaction Setup</th><th colspan="4">qPCR Program</th><th>QR Code for Thermal Protocol</th></tr>
<tr><th colspan="4">CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad) and Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)</th><td rowspan="11">  <br>https://www.bioeksen.com.tr/files/bacillus_anthracis</td></tr>
<tr><th>Reagent</th><th>Volume/Rxn</th><th>Step</th><th>Cycle No.</th><th>Temperature</th><th>Duration</th></tr>
<tr><td rowspan="2">2X qPCR Mix</td><td rowspan="2">5 µL</td><td>Reverse Transcription</td><td>1 Cycle</td><td>52 °C</td><td>3 min</td></tr>
<tr><td>Pre-Incubation</td><td>1 Cycle</td><td>95 °C</td><td>10 sec</td></tr>
<tr><td rowspan="2">BA Oligo Mix</td><td rowspan="2">2.5 µL</td><td>Denaturation</td><td rowspan="2">12 Touch Down Cycles:<br>1 °C decrement in annealing temperature per cycle</td><td>95 °C</td><td>1 sec</td></tr>
<tr><td>Annealing and Extension</td><td>67 °C – 56 °C</td><td>10 sec</td></tr>
<tr><td rowspan="2">Template Nucleic Acid</td><td rowspan="2">2.5 µL</td><td>Denaturation</td><td rowspan="3">35 Cycles</td><td>85 °C</td><td>1 sec</td></tr>
<tr><td>Annealing and Extension</td><td>55 °C</td><td>10 sec</td></tr>
<tr><td>Total Reaction Volume</td><td>10 µL</td><td>Detection (Reading)</td><td colspan="2">(FAM-Green)/(HEX-Yellow)</td></tr>
</table>

**WARNING: The qPCR program template of the instrument's software must be downloaded from the manufacturer's website to avoid errors in the manual entry.**

## 9. Interpretation of the Assay Results

- All default analysis options (e.g. **auto-calculated threshold**) in the related software of ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System – BMS)*** instruments should not be changed to calculate Cq values.
- Shape of the amplification curves obtained in the FAM/HEX channels should be examined for all reaction wells returning with Cq values. All the **sigmoidal curves above the threshold** should be recorded as "**positive**" and their Cq values should be recorded. **Non-sigmoidal curves** should be recorded as "**negative**".
- For samples with a sigmoidal curve below the auto-calculated threshold for the ***CFX96 Touch™/CFX96™ Dx (Bio-Rad) and CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments, **the threshold level should be manually set to 200 RFU. If the sigmoidal curve exceeds the threshold, the Ct value should be recorded as "35"** and the sample should be reported as "**positive**".
- For **samples with a sigmoidal curve below the auto-calculated threshold** for ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** should be reported as "**positive**" and **their Cq values should be recorded as "35"**.

**Table 5. Expected Performance of the Kit Controls**

| Control Type | Control Name | Purpose | Expected Results and Cq Values | |
|---|---|---|---|---|
| | | | ***Internal Control* (HEX)** | ***Target* (FAM)** |
| **Negative Control** | NTC | Contamination control during qPCR | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **No template addition** | NRC | Reagent contamination control | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **Positive Control** | PC | Reagent integrity | **Detected** (Cq≤33) | **Detected** (Cq≤33) |
| **Internal/Extraction Control** | IC | To monitor the integrity of nucleic acid extraction and qPCR from each sample | **Detected** (Cq≤33)<br>If IC Cq>33 check the target Cq | If target Cq≤35.0, conclude it as IC is valid |

If any control does not perform as described above, the run is considered invalid, and the test is repeated.

1. **Invalid PC (Cq>33 in any channel):** It is recommended to contact the manufacturer, renew the reagents, and repeat the reaction.
2. **Invalid NTC (No Cq in any channel):** Repeat the analysis by paying attention to the "**Warnings**" section.
3. **Invalid NRC (No Cq in any channel):** Contact the manufacturer, renew the reagents, and repeat the reaction.
4. **Invalid IC (Cq>33 in HEX channel and no Cq in the other channels):** Repeat the analysis. If the problem continues, then conclude it as an invalid PCR template.

If all the controls are valid, proceed to the interpretation of the results.

**Table 6. Interpretation of Patient Samples**

| Target | Internal Control | Results Interpretation | Action |
|---|---|---|---|
| **Positive (+)** | **Positive (+)** | Results are VALID,<br>Target DNA is detected | Report it as **POSITIVE** |
| **Positive (+)** | **Negative (-)** | Results are VALID,<br>Target DNA is detected | Report it as **POSITIVE** |
| **Negative (-)** | **Positive (+)** | Results are VALID,<br>Target DNA is not detected | Report it as **NEGATIVE** |
| **Negative (-)** | **Negative (-)** | Results are INVALID<br>(sampling/extraction/inhibition problem) | Re-extract the specimen and perform testing again. If the result is still invalid, a new specimen should be obtained. If an additional clinical sample is unavailable, report it as **INVALID** |

**WARNING: On the web page linked with the QR code, examples of the sigmoidal amplification curves are given. The results obtained with this kit should NOT be interpreted without examining these samples.**

## 10. Limitations

- ***Bio-Speedy® Bacillus anthracis Real-Time PCR Detection Kit*** is intended for use by laboratory personnel trained in the techniques of qPCR and *in vitro* diagnostic procedures.
- The clinical specimens shall be collected by a healthcare provider in accordance with the specimen collection guidelines.
- A false-negative result may occur if a specimen is improperly collected, transported, or handled.
- Mutations within the target regions of the ***Bio-Speedy® Bacillus anthracis Real-Time PCR Detection Kit*** could affect primer and/or probe binding resulting in failure to detect the presence of bacteria.
- Inhibitors or other types of interference may produce a false-negative result. False-negative results may also occur if inadequate numbers of organisms are present in the specimen.

## 11. Explanation of Symbol

| Symbol | Title of Symbol | Symbol | Title of Symbol | Symbol | Title of Symbol |
|---|---|---|---|---|---|
| CE | European Conformity CE Mark | LOT | Batch code | Σ | Contains sufficient for *<n>* tests |
| IVD | *In vitro* diagnostic *medical device* | REF | Catalogue number | | Keep away from sunlight |
| | Manufacturer | NON STERILE | Non-*sterile* | | Protect from heat and radioactive sources |
| | Use-by date | | Consult *instructions for use* or consult electronic *instructions for use* | | Do not use if package is damaged and consult *instructions for use* |
| CONTROL - | Negative control | | Caution | | Keep dry |
| CONTROL + | Positive control | | Temperature limit | | Keep it upright |
| CONTROL | Control | | | | |

## 12. Manufacturer and Technical Support

**Bioeksen AR GE Teknolojileri A.Ş.**
**Address:** Huzur Mah. Metin Oktay Cad. Nurol Life Sitesi D Blok No:3/31, 34396 Sarıyer/İstanbul-TÜRKİYE
**Phone:** +90 (212) 285 10 17, **Fax:** +90 (212) 285 10 18
**Web**: www.bioeksen.com.tr, E**-mail:** info@bioeksen.com.tr,
**Technical Support**: support@bioeksen.com.tr

**Notice to User:** Please inform us about product-related incidents at "**vigilance@bioeksen.com.tr**" within 24 hours.

*For in vitro diagnostic use only.*
*For laboratory professional use only.*

**Cat No:** BS-LP-25/BS-LP-100

# Legionella pneumophila qPCR Kit

## Package Insert

### 1. Kit Content

**Table 1. Kit Content**

| Component | Intended Use | Amount (10 µL/Rxn) | |
|---|---|---|---|
| | | 25 Rxns | 100 Rxns |
| 2X qPCR Mix | Optimized ready-to-use mix for qPCR assay | 1 x 125 µL | 1 x 500 µL |
| LP Oligo Mix | Specific nucleic acid amplification and detection:<br>FAM: *Legionella pneumophila mip* gene<br>HEX: Human genome *RNase P* as an internal control | 1 x 62.5 µL | 1 x 250 µL |
| NTC | Negative (No Template) Control<br>(Nuclease-Free Water) | 1 x 1000 µL | 1 x 1000 µL |
| PC-LP | Positive Control (Synthetic DNA fragment mixture of the targets in the "LP Oligo Mix") | 1 x 100 µL | 1 x 250 µL |

**Table 2. Storage Requirements and Shelf Life**

| Component | Transport Condition | Storage Condition | Shelf Life |
|---|---|---|---|
| 2X qPCR Mix | -22 °C to +8 °C | -22 °C to -18 °C | 12 months |
| LP Oligo Mix | | -22 °C to -18 °C | |
| NTC | | -22 °C to -18 °C / +2 °C to +8 °C | |
| PC-LP | | -22 °C to -18 °C before opening, +2 °C to +8 °C after the first thaw | |

**! Each reagent stored at storage temperature can be used until the expiration date indicated on the tube following the first opening. The expiration date of the kit is determined by the expiration date of the reagents.**

### 2. Materials Required but Not Provided

**Table 3. Components Required but Not Included with The Test**

| Components Required but Not Included with The Test | |
|---|---|
| **1.** Real-Time PCR instrument with FAM and HEX channels Ramp rate ≥3 °C/sec | **7.** Reaction tubes and their caps/seals compatible with the qPCR instruments and the reaction volume |
| **2.** Adjustable micropipettes and compatible pipette tips (nuclease-free) | **Extra components recommended to use:** |
| **3.** Centrifuge | **8.** Biosafety cabinet for PCR setup |
| **4.** Vortex | **9.** Cold tube rack (for microcentrifuge tubes and PCR tubes/strips) |
| **5.** Nuclease-free water/viral transport medium/serum physiologic | **10.** PPE (Personal Protective Equipment) |
| **6.** 1.5- or 2-mL microcentrifuge tubes (nuclease-free) | |

### 3. Intended Use and Test Principle

Aerobic gram-negative bacteria called *Legionellae* are linked to respiratory infections. There are currently around 50 different *Legionella* species and 70 different serogroups, several of which may infect humans and cause an infection. The most prevalent pathogenic species, *Legionella pneumophila*, has at least 16 distinct serogroups. At an American Legion convention in Philadelphia in 1976, a pneumonia outbreak led to the discovery of Legionella pneumophila as a disease pathogen. Up to 90% of cases of legionellosis, including Pontiac fever and legionnaires' disease (LD), are caused by this species. The initial symptom of legionnaires' illness is severe multisystem pneumonia. Increased death rates among the elderly and patients with serious underlying diseases could be led on by diagnostic delays.

***Bio-Speedy® Legionella pneumophila qPCR Kit*** is a one-step real-time PCR (qPCR) test intended for the qualitative detection of the DNA from *Legionella pneumophila*. The ***Bio-Speedy® Legionella pneumophila qPCR Kit*** is applied to nucleic acids obtained from anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples. The kit is non-automated and functions as an aid to diagnosis.

Detection with the kit is achieved via rapid nucleic acid extraction from anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples followed by multiplex qPCR targeting the *Legionella pneumophila* specific *macrophage infectivity potentiator* ***(mip)*** gene in real-time PCR instruments that are equipped with FAM and HEX detection channels. **The kit allows to achieve qPCR result in 43 minutes (Run time may vary depending on the instrument)**.

The oligonucleotide set targeting human ***RNase P*** mRNA functions as a control of the sampling, nucleic acid extraction, and qPCR since the oligonucleotide set targets the exon-exon junction. The kit also contains negative and positive control templates for testing the contamination and the qPCR reagent stability, respectively.

***Bio-Speedy® Legionella pneumophila qPCR Kit*** is intended for use by laboratory personnel trained in the techniques of qPCR and *in vitro* diagnostic procedures.

### 4. Analytical Specifications

***Bio-Speedy® Legionella pneumophila qPCR Kit*** is validated with ***Bio-Speedy® νNAT® Transfer Tube*** *(Catalog No: BS-NA-513-100),* ***νNAT® Transfer Tube*** *(Catalog No: BS-NA-513m-100),* ***Bio-Speedy® νNAT® Viral Nucleic Acid Buffer*** *(Catalog No: BS-NA-510-100/BS-NA-510-250/BS-NA-510-500/BS-NA-510-1000),* and ***Bio-Speedy® 5min NA*** *(Catalog No: BS-NA-514-100/BS-NA-514-250/BS-NA-514-500/BS-NA-514-1000)* for nucleic acids prepared from anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples.

The qPCR is carried out in 10 µL reaction volume using the ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** Real-Time PCR systems equipped with the FAM and HEX detection channels.

For the analysis performed on *Bio-Rad Real-Time PCR systems*, the kit has been validated with white reaction tubes specific to these systems. **The clear reaction tubes result in 5-10 times lower fluorescence signal in the *Bio-Rad* instruments compared to the white tubes.** Besides, device-specific reaction tubes should be used on ***BMS*** instrument (the specified analytical performance of the kit can only be achieved using the validated tubes).

Limit of Detection (LoD) of the ***Bio-Speedy® Legionella pneumophila qPCR Kit*** is determined as 54 cp/mL for *Legionella pneumophila*.

The exclusivity of the kit was tested on different pathogens. No cross-reaction was observed in analytical specificity studies performed on reference strains and field isolates. The sensitivity and specificity of the kit were determined as 100.00% and 100.00%, respectively.

## 5. Collection, Storage and Shipment of Clinical Specimens

Clinical samples (anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples) are collected from individuals by a healthcare provider in accordance with the specimen collection guidelines. Anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, and oral/saliva swab samples are transferred into the ***Bio-Speedy® νNAT® Transfer Tube*** *(Catalog No: BS-NA-513-100)* or ***νNAT® Transfer Tube*** *(Catalog No: BS-NA-513m-100)* containing 2 mL of the ***νNAT® reagent*** or into a sterile transport tube containing 3 mL of Viral Transport Medium (VTM) (Preparation of Viral Transport Medium, Centers for Disease Control and Prevention, SOP#: DSR-052-05). Bronchoalveolar lavage and nasopharyngeal aspirate samples should be transferred into sterile containers containing 3 mL of VTM. Other sample types (saliva, gargle, and sputum samples) should be transferred into preservative-free sterile tubes.

Specimens should be sent to the laboratory within 4 hours after collection at 2 °C to 8 °C. If a delay in delivery for more than 3 days is expected, the samples should be frozen at -70 °C and delivered with dry ice. It is important that the samples should not be exposed to repeated freeze-thaw.

Specimens in the VTM can be stored at 2 °C to 8 °C for up to 72 hours, and specimens in the ***νNAT® Transfer Tube*** or ***Bio-Speedy® νNAT® Transfer Tube*** can be stored at 2 °C to 8 °C for up to 3 months. If a delay in the qPCR test is expected, specimens can be stored at -70 °C. If not available, specimens can be stored at -20 °C.

## 6. Preparation of Nucleic Acid Samples

One minute after the collection, the swab samples in the ***Bio-Speedy® νNAT® Transfer Tube*** or ***νNAT® Transfer Tube*** can be used directly in qPCR.

The dry swab samples are combined with 1 mL of "molecular grade water: ***Bio-Speedy® νNAT® Viral Nucleic Acid Buffer (10X concentrated)*** (in a 9:1 ratio)" mixture. The mixture containing the swab can be used directly in qPCR after 1 minute of incubation at room temperature.

The samples in the VTM or the saline are combined with the ***Bio-Speedy® νNAT® Viral Nucleic Acid Buffer (10X concentrated)*** in a 9:1 (sample: ***νNAT®***) ratio. The mixture can directly be used in qPCR after 1 minute of incubation at room temperature.

***Bio-Speedy® 5min NA*** is used according to the manufacturer's instructions.

The samples are combined with the ***5min NA reagent*** in a 19:1 (Sample:***5min NA***) ratio (e.g., 47.5 µL Sample:2.5 µL ***5min NA***). The mixture is incubated in a thermal cycler for 2 min at 65 °C, 3 min at 95 °C, and 1 sec at 40 °C. Afterward, the samples are ready to use in the qPCR reaction.

## 7. Warnings

1. Specimen processing should be performed in accordance with national biological safety recommendations.
2. Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
3. All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate. Sampling should be carried out by personnel with sufficient knowledge and experience.
4. The kit should be stored away from nucleic acid sources and PCR amplicons.
5. Except for fluid transfers, nucleic acid, and positive control tubes should always be kept closed.
6. To prevent contamination of the reaction mixture by previously amplified target sequences, maintain separate work areas, and dedicated equipment.
7. Different sets of laboratory coats should be worn pre- and post-PCR.
8. The micropipettes used for pipetting PCR mixes and template nucleic acids should be separate. Filtered and nuclease-free tips should be used.
9. Cotton or calcium alginate swabs or swabs with wooden sticks should not be used since they may contain substances that inactivate some pathogens and inhibit PCR.
10. It is recommended to use swabs with the breakable shaft to prevent contamination during sampling.
11. The components in the kit should not be mixed with different lot numbers or chemicals of the same name but from different manufacturers.
12. Master stock reagents should be kept on the cold block during the PCR setup.
13. Kit components should be mixed by gently shaking before use.
14. Maintenance/calibration interval should be determined for all instruments and equipment used with the kit.
15. To avoid false positives due to amplified material, the PCR-completed reaction tubes should be disposed of before opening in the laboratory.
16. The wipeable surfaces of the rooms, benches and devices should be cleaned regularly with freshly diluted 10% bleach solution (0.5% NaClO).
17. Dispose of waste in a designated matter in accordance with local, regional, and federal regulations.

## 8. qPCR Application Protocol

**Table 4. Reaction Set-up and qPCR Program Details**

| Reaction Setup | | qPCR Program | | | | QR Code for Thermal Protocol |
|---|---|---|---|---|---|---|
| | | *CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad) and Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)* | | | | |
| **Reagent** | **Volume/Rxn** | **Step** | **Cycle No.** | **Temperature** | **Duration** | |
| 2X qPCR Mix | 5 µL | **Reverse Transcription** | 1 Cycle | 52 °C | 3 min | |
| | | **Pre-Incubation** | 1 Cycle | 95 °C | 10 sec | |
| LP Oligo Mix | 2.5 µL | **Denaturation** | 12 Touch Down Cycles: 1 °C decrement in annealing temperature per cycle | 95 °C | 1 sec | |
| | | **Annealing and Extension** | | 67 °C – 56 °C | 10 sec | |
| Template Nucleic Acid | 2.5 µL | **Denaturation** | 35 Cycles | 85 °C | 1 sec | |
| | | **Annealing and Extension** | | 55 °C | 10 sec | |
| **Total Reaction Volume** | **10 µL** | **Detection (Reading)** | | (FAM-Green)/(HEX-Yellow) | | https://www.bioeksen.com.tr/files/legionella_pneumophila |

**WARNING: The qPCR program template of the instrument's software must be downloaded from the manufacturer's website to avoid errors in the manual entry.**

## 9. Interpretation of the Assay Results

- All default analysis options (e.g. **auto-calculated threshold**) in the related software of ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System – BMS)*** instruments should not be changed to calculate Cq values.
- Shape of the amplification curves obtained in the FAM/HEX channels should be examined for all reaction wells returning with Cq values. All the **sigmoidal curves above the threshold** should be recorded as "**positive**" and their Cq values should be recorded. **Non-sigmoidal curves** should be recorded as "**negative**".
- For samples with a sigmoidal curve below the auto-calculated threshold for the ***CFX96 Touch™/CFX96™ Dx (Bio-Rad) and CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments, **the threshold level should be manually set to 200 RFU**. **If the sigmoidal curve exceeds the threshold, the Ct value should be recorded as "35"** and the sample should be reported as "**positive**".
- For **samples with a sigmoidal curve below the auto-calculated threshold** for ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** should be reported as "**positive**" and **their Cq values should be recorded as "35"**.

**Table 5. Expected Performance of the Kit Controls**

| Control Type | Control Name | Purpose | Expected Results and Cq Values | |
|---|---|---|---|---|
| | | | *Internal Control* (HEX) | *Target* (FAM) |
| **Negative Control** | NTC | Contamination control during qPCR | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **No template addition** | NRC | Reagent contamination control | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **Positive Control** | PC | Reagent integrity | **Detected** (Cq≤33) | **Detected** (Cq≤33) |
| **Internal/Extraction Control** | IC | To monitor the integrity of nucleic acid extraction and qPCR from each sample | **Detected** (Cq≤33) If IC Cq>33 check the target Cq | If target Cq≤35.0, conclude it as IC is valid |

If any control does not perform as described above, the run is considered invalid, and the test is repeated.

1. **Invalid PC (Cq>33 in any channel):** It is recommended to contact the manufacturer, renew the reagents, and repeat the reaction.
2. **Invalid NTC (No Cq in any channel):** Repeat the analysis by paying attention to the "**Warnings**" section.
3. **Invalid NRC (No Cq in any channel):** Contact the manufacturer, renew the reagents, and repeat the reaction.
4. **Invalid IC (Cq>33 in HEX channel and no Cq in the other channels):** Repeat the analysis. If the problem continues, then conclude it as an invalid PCR template.

If all the controls are valid, proceed to the interpretation of the results.

**Table 6. Interpretation of Patient Samples**

| Target | Internal Control | Results Interpretation | Action |
|---|---|---|---|
| **Positive (+)** | **Positive (+)** | Results are VALID, Target DNA is detected | Report it as **POSITIVE** |
| **Positive (+)** | **Negative (-)** | Results are VALID, Target DNA is detected | Report it as **POSITIVE** |
| **Negative (-)** | **Positive (+)** | Results are VALID, Target DNA is not detected | Report it as **NEGATIVE** |
| **Negative (-)** | **Negative (-)** | Results are INVALID (sampling/extraction/inhibition problem) | Re-extract the specimen and perform testing again. If the result is still invalid, a new specimen should be obtained. If an additional clinical sample is unavailable, report it as **INVALID** |

**WARNING: On the web page linked with the QR code, examples of the sigmoidal amplification curves are given. The results obtained with this kit should NOT be interpreted without examining these samples.**

## 10. Limitations

- ***Bio-Speedy® Legionella pneumophila qPCR Kit*** is intended for use by laboratory personnel trained in the techniques of qPCR and *in vitro* diagnostic procedures.
- The clinical specimens shall be collected by a healthcare provider in accordance with the specimen collection guidelines.
- A false-negative result may occur if a specimen is improperly collected, transported, or handled.
- The use of cotton or calcium alginate swabs or swabs with wooden sticks can lead to false-negative results since they may contain substances that inactivate some pathogens and inhibit PCR.
- Mutations within the target regions of the ***Bio-Speedy® Legionella pneumophila qPCR Kit*** could affect primer and/or probe binding resulting in failure to detect the presence of bacteria.
- Inhibitors or other types of interference may produce a false-negative result. False-negative results may also occur if inadequate numbers of organisms are present in the specimen.

## 11. Explanation of Symbol

| Symbol | Title of Symbol | Symbol | Title of Symbol | Symbol | Title of Symbol |
|---|---|---|---|---|---|
| CE | European Conformity CE Mark | LOT | Batch code | Σ | Contains sufficient for <*n*> tests |
| IVD | *In vitro* diagnostic *medical device* | REF | Catalogue number | | Keep away from sunlight |
| | Manufacturer | NON STERILE | Non-*sterile* | | Protect from heat and radioactive sources |
| | Use-by date | | Consult *instructions for use* or consult electronic *instructions for use* | | Do not use if package is damaged and consult *instructions for use* |
| CONTROL - | Negative control | | Caution | | Keep dry |
| CONTROL + | Positive control | | Temperature limit | | Keep it upright |
| CONTROL | Control | | | | |

## 12. Manufacturer and Technical Support

**Bioeksen R&D Technologies Incorporated Company**
**Address:** Resitpasa Mh. Katar Cd., 4/B-105. 34467, Sariyer, Istanbul, TURKEY.
**Phone:** +90 (212) 285 10 17, **Fax:** +90 (212) 285 10 18
**Web**: www.bioeksen.com.tr, E-**mail:** info@bioeksen.com.tr,
**Technical Support**: support@bioeksen.com.tr

**Notice to User:** Please inform us about product-related incidents at "**vigilance@bioeksen.com.tr**" within 24 hours.

*For in vitro diagnostic use only*
*For laboratory professional use only.*

**Cat No:** BS-SY-MX24T-25/BS-SY-MX24T-100

# Respiratory Tract RT-qPCR MX-24T Panel

## Package Insert

### 1. Kit Content

**Table 1. Kit Content**

| Oligo Mix Content | | | | | Positive Control Content | | |
|---|---|---|---|---|---|---|---|
| **Component** | **Target** | **Channel** | **Quantity (20 µL/Rxn) 25 Rxns** | **Quantity (20 µL/Rxn) 100 Rxns** | **Component** | **Quantity (20 µL/Rxn) 25 Rxns** | **Quantity (20 µL/Rxn) 100 Rxns** |
| COVID/Flu Oligo Mix | SARS-CoV-2 | FAM | 1 x 125 µL | 1 x 500 µL | PC-COVID/Flu | 1 x 100 µL | 1 x 100 µL |
| | Internal Control (Human *RNase P* gene) | HEX | | | | | |
| | Influenza B | ROX | | | | | |
| | Influenza A | CY5 | | | | | |
| COR Oligo Mix | Human Corona 229E | FAM | 1 x 125 µL | 1 x 500 µL | PC-COR | 1 x 100 µL | 1 x 100 µL |
| | Human Corona OC43 | HEX | | | | | |
| | Human Corona NL63 | ROX | | | | | |
| | Human Corona HKU1 | CY5 | | | | | |
| PAR Oligo Mix | Human Parainfluenza 1 | FAM | 1 x 125 µL | 1 x 500 µL | PC-PAR | 1 x 100 µL | 1 x 100 µL |
| | Human Parainfluenza 2 | HEX | | | | | |
| | Human Parainfluenza 3 | ROX | | | | | |
| | Human Parainfluenza 4 | CY5 | | | | | |
| MEA Oligo Mix | Human Metapneumovirus | FAM | 1 x 125 µL | 1 x 500 µL | PC-MEA | 1 x 100 µL | 1 x 100 µL |
| | Enterovirus/Human Rhinovirus Oligo Set 1 | HEX | | | | | |
| | - | ROX | | | | | |
| | Adenovirus | CY5 | | | | | |
| BPR Oligo Mix | Human Bocavirus | FAM | 1 x 125 µL | 1 x 500 µL | PC-BPR | 1 x 100 µL | 1 x 100 µL |
| | - | HEX | | | | | |
| | Human Parechovirus | ROX | | | | | |
| | Enterovirus/Human Rhinovirus Oligo Set 2 | CY5 | | | | | |
| LMC Oligo Mix | *Legionella pneumophila* | FAM | 1 x 125 µL | 1 x 500 µL | PC-LMC | 1 x 100 µL | 1 x 100 µL |
| | - | HEX | | | | | |
| | *Mycoplasma pneumoniae* | ROX | | | | | |
| | *Chlamydophila pneumoniae* | CY5 | | | | | |
| HBS Oligo Mix | *Haemophilus influenzae* | FAM | 1 x 125 µL | 1 x 500 µL | PC-HBS | 1 x 100 µL | 1 x 100 µL |
| | - | HEX | | | | | |
| | *Bordetella pertussis* | ROX | | | | | |
| | *Streptococcus pneumoniae* | CY5 | | | | | |
| RSV Oligo Mix | Respiratory syncytial virus A/B | FAM | 1 x 125 µL | 1 x 500 µL | PC-RSV | 1 x 100 µL | 1 x 100 µL |
| | - | HEX | | | | | |
| | - | ROX | | | | | |
| | - | CY5 | | | | | |

| Component | Intended Use | 25 Rxns | 100 Rxns |
|---|---|---|---|
| 2X Prime Script Mix | Optimized ready-to-use mix for RT-qPCR assay | 2 x 1000 µL | 7 x 1250 µL |
| NTC | Negative (No Template) Control (Nuclease-free Water) | 1 x 1000 µL | 1 x 1000 µL |

**Table 2. Storage Requirements and Shelf Life**

| Component | Transport Condition | Storage Condition | Shelf Life |
|---|---|---|---|
| 2X Prime Script Mix | -22 °C to +8 °C | -22 °C to -18 °C | 12 Months |
| Oligo Mix | | -22 °C to -18 °C | |
| NTC | | -22 °C to -18 °C / +2 °C to +8 °C | |
| PC | | -22 °C to -18 °C before opening, +2 °C to +8 °C after first thaw | |

**Each reagent stored at storage temperature can be used until the expiration date indicated on the tube following the first opening. The expiration date of the kit is determined by the expiration date of the reagents.**

### 2. Materials Required but Not Provided

**Table 3. Components Required but Not Included with The Test**

| Components Required but Not Included with The Test | |
|---|---|
| **1.** Real-Time PCR instrument with FAM, HEX, ROX, and CY5 channels, Ramp rate ≥3 °C/sec | **7.** Reaction tubes and their caps/seals compatible with the qPCR instruments and the reaction volume |
| **2.** Adjustable micropipettes and compatible pipette tips (nuclease-free) | **Extra components recommended to use:** |
| **3.** Centrifuge | **8.** Biosafety cabinet for PCR setup |
| **4.** Vortex | **9.** Cold tube rack for microcentrifuge tubes and PCR tubes/strips |
| **5.** Nuclease-free water/viral transport medium/serum physiologic | **10.** PPE (Personal Protective Equipment) |
| **6.** 1.5- or 2-mL microcentrifuge tubes (nuclease-free) | |

## 3. Intended Use and Test Principle

***Bio-Speedy® Respiratory Tract RT-qPCR MX-24T Panel*** is a one-step reverse transcription and real-time PCR (RT-qPCR) test intended for the presumptive qualitative detection of the viral and bacterial agents given in Table 1. The ***Bio-Speedy® Respiratory Tract RT-qPCR MX-24T Panel*** is applied to nucleic acids obtained from combined nasopharyngeal and oropharyngeal swab, bronchoalveolar lavage, nasopharyngeal aspirate, and sputum samples.

Detection with the kit is achieved via rapid nucleic acid extraction from respiratory tract samples followed by multiplex RT-qPCR targeting the genomic RNA and DNA regions specific to the target agents in real-time PCR instruments that are equipped with **FAM**, **HEX**, **ROX**, and **CY5** detection channels. **The kit allows to achieve RT-qPCR result in 60 minutes. (Run time may vary depending on the instrument)**

The oligonucleotide set targeting the human ***RNase P*** mRNA functions as a control of the sampling, nucleic acid extraction, reverse transcription, and qPCR since the oligonucleotide set targets the exon-exon junction. The kit also contains negative and positive control templates for testing the contamination and the RT-qPCR reagent stability, respectively.

***Bio-Speedy® Respiratory RT-qPCR MX-24T Panel*** is intended for use by laboratory personnel trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures.

## 4. Analytical Specifications

***Bio-Speedy® Respiratory Tract RT-qPCR MX-24T Panel*** is validated with ***Bio-Speedy® νNAT® Transfer Tube*** *(Catalog No: BS-NA-513-100)* and ***νNAT® Transfer Tube*** *(Catalog No: BS-NA-513m-100)* for combined nasopharyngeal and oropharyngeal swab samples.

The kit is validated with ***Bio-Speedy® νNAT® Viral Nucleic Acid Buffer*** *(Catalog No: BS-NA-510-100/BS-NA-510-250/BS-NA-510-500/BS-NA-510-1000)* for combined nasopharyngeal and oropharyngeal swab samples in VTM.

The kit is validated with ***Bio-Speedy® 5min NA*** *(Catalog No: BS-NA-514-100/BS-NA-514-250/BS-NA-514-500/BS-NA-514-1000)* and ***Zybio EXM3000 Nucleic Acid Isolation System*** *(Model No: EXM3000)* for bronchoalveolar lavage, nasopharyngeal aspirate, and sputum samples.

Limit of Detection (LoD) of the kit is between 125-500 copies/mL for combined nasopharyngeal and oropharyngeal swab samples in the ***Bio-Speedy® νNAT® Transfer Tube***, 250-1000 copies/mL for combined nasopharyngeal and oropharyngeal swab samples in the VTM extracted using the ***Bio-Speedy® νNAT® Viral Nucleic Acid Buffer,*** 250-1000 copies/mL for bronchoalveolar lavage, nasopharyngeal aspirate and sputum samples in the prepared using the ***Bio-Speedy® 5min NA*** and 250-1000 copies/mL for bronchoalveolar lavage, nasopharyngeal aspirate and sputum samples using the ***Zybio EXM3000 Nucleic Acid Isolation System***.

**Table 4. Summary of LoD Results Based on The Specimen Type and Extraction Method**

| NO | Specimen Type | Sample Transfer Method | | Extraction Method | | | LoD (cp/mL) |
|---|---|---|---|---|---|---|---|
| | | VTM | *νNAT® Transfer Tube* | *Bio-Speedy® νNAT® Viral Nucleic Acid Buffer* | *Bio-Speedy® 5min NA* | *Zybio EXM3000 Nucleic Acid Isolation System* | |
| 1 | Combined nasopharyngeal and oropharyngeal swab | - | ✓ | - | | | 125-500 |
| 2 | Combined nasopharyngeal and oropharyngeal swab | ✓ | - | ✓ | - | - | 250-1000 |
| 3 | Bronchoalveolar lavage, nasopharyngeal aspirate, and sputum | - | | - | ✓ | - | 250-1000 |
| 4 | Bronchoalveolar lavage, nasopharyngeal aspirate, and sputum | - | | - | - | ✓ | 250-1000 |

The RT-qPCR is carried out in 20 µL reaction volume using the ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System – BMS)*** Real-Time PCR detection systems equipped with the **FAM**, **HEX**, **ROX**, and **CY5** detection channels.

The exclusivity of the kit was tested on 43 different viral and bacterial strains and a pool of nasal washes from 20 different healthy people. The kit does not cross-react with other respiratory pathogens and human respiratory microbial flora. The sensitivity and specificity of the kit were determined as 98.95% and 99.13%, respectively.

## 5. Collection, Storage, and Shipment of Clinical Specimens

Clinical samples (combined nasopharyngeal and oropharyngeal swab, bronchoalveolar lavage, nasopharyngeal aspirate, and sputum samples) are collected from individuals by a healthcare provider in accordance with the specimen collection guidelines. The swab samples are transferred into the ***Bio-Speedy® νNAT® Transfer Tube*** (*Catalog No: BS-NA-513-100*) containing 2 mL of the ***νNAT® reagent*** or into a sterile transport tube containing 3 mL of Viral Transport Medium (VTM) (Preparation of Viral Transport Medium, Centers for Disease Control and Prevention, SOP#: DSR-052-05 without antibiotics). Bronchoalveolar lavage, nasopharyngeal aspirate, and sputum samples should be transferred into sterile containers.

Clinical samples are transported and stored in accordance with the specimen labeling, storage & handling guidelines. The specimens in the ***Bio-Speedy® νNAT® Transfer Tube*** can be stored and transferred to the laboratory at room temperature within 24 hours. For transfers longer than 24 hours, ship the specimens to the laboratory on ice pack. If a specimen is frozen at -70 °C or lower, ship overnight to the laboratory on dry ice. It is important that specimens are not exposed to continuous freeze-thaw exposure. Store the specimens in the VTM or preservative-free sterile containers at 2 °C to 8 °C and ship them to the laboratory on an ice pack.

After collection, specimens in the VTM or preservative-free sterile containers can be stored at 2 °C to 8 °C for up to 72 hours, and specimens in the ***Bio-Speedy® νNAT®Transfer Tube*** can be stored at 2 °C to 8 °C for up to 3 months. If a delay in the RT-qPCR test is expected, store the specimens at -70 °C or lower.

## 6. Warnings

1. Specimen processing should be performed in accordance with national biological safety recommendations.
2. Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
3. All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate. Sampling should be carried out by personnel with sufficient knowledge and experience.
4. The kit should be stored away from nucleic acid sources and PCR amplicons.
5. Except for fluid transfers, nucleic acid, and positive control tubes should always be kept closed.
6. To prevent contamination of the reaction mixture by previously amplified target sequences, maintain separate work areas and dedicated equipment.
7. Different sets of laboratory coats should be worn in pre- and post-PCR areas.
8. The micropipettes used for pipetting PCR mixes and template nucleic acids should be separate. Filtered nuclease-free tips should be used.

9. Cotton or calcium alginate swabs or swabs with wooden sticks should not be used since they may contain substances that inactivate some pathogens and inhibit PCR.
10. It is recommended to use swabs with breakable shafts to prevent contamination during sampling.
11. The components in the kit should not be mixed with different lot numbers or chemicals of the same name but from different manufacturers.
12. Master stock reagents should be kept on the cold block during the PCR setup.
13. Kit components should be mixed by gently shaking before use.
14. Maintenance/calibration interval should be determined for all instruments and equipment used with the kit.
15. Immediately after each RT-qPCR run, dispose of the qPCR tubes in closed bags to avoid PCR amplicon contamination in the lab.
16. The wipeable surfaces of the rooms, benches, and devices should be cleaned regularly with freshly diluted 10% bleach solution (0.5% NaClO).
17. Dispose of waste in a designated matter in accordance with local, regional, and federal regulations.

## 7. RT-qPCR Application Protocol

Before starting the assay, please consider the following:

1. The kit was validated only for the template nucleic acid volume that is 25% of the total RT-qPCR volume.
2. The kit cannot be used with real-time PCR instruments without periodic maintenance records.
3. **It is recommended to use a validated qPCR plate/strip with the kit!** The specified analytical performance of the kit can only be achieved using the validated tubes.
4. **For testing the contamination, set up two different negative control reactions with and without the addition of NTC.**

Program the qPCR device as follows and add the reagents into the qPCR tubes, close the tubes, place them into the qPCR instrument and start the run (Table 5).

**Table 5. Real-Time PCR Program**

| Reaction Setup | | RT-qPCR Program | | | | QR Code for Thermal Protocols |
|---|---|---|---|---|---|---|
| | | *CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)* and *Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)* | | | | |
| **Reagent** | **Volume/Rxn** | **Step** | **Cycle No.** | **Temperature** | **Duration** | |
| 2X Prime Script Mix | 10 µL | **Reverse Transcription** | 1 Cycle | 52 °C | 5 min | |
| | | **Pre-Incubation** | 1 Cycle | 95 °C | 10 sec | |
| Oligo Mix | 5 µL | **Denaturation** | 12 Touch Down Cycles: 1 °C decrement in annealing temperature per cycle | 95 °C | 1 sec | |
| | | **Annealing and Extension** | | 67 °C to 56 °C | 30 sec | |
| Template Nucleic Acid | 5 µL | **Denaturation** | 35 Cycles | 95 °C | 1 sec | |
| | | **Annealing and Extension** | | 55 °C | 30 sec | |
| **Total Reaction Volume** | **20 µL** | **Detection (Reading)** | | *(FAM-Green)/(HEX-Yellow)/(ROX-Orange)/(CY5-Red)* | | www.bioeksen.com.tr/files/respiratory_tract_mx-24t_panel/ |

## 8. Interpretation of the Assay Results

- Manually adjust the threshold level to **1500 RFU**, other default analysis options in the related software of ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments should not be changed to calculate Cq values.
- All default analysis options (e.g. **auto-calculated threshold**) in the related software of ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS*** instruments should not be changed to calculate Cq values.
- Shape of the amplification curves obtained in the FAM/HEX/ROX/CY5 channels should be examined for all reaction wells returning with Cq values. All the **sigmoidal curves above the threshold** should be recorded as "**positive,**" and their Cq values should be recorded. **Non-sigmoidal curves** should be recorded as "**negative**".

When **more than one parameter is positive** in a respiratory sample, final reporting is performed after the following evaluation process:

1. The parameter giving the lowest Cq is determined = Min Cq
2. (Cq value of other parameter) – (Min Cq) If <7, **positive** result is given for other parameter.
3. (Cq value of other parameter) – (Min Cq) If ≥7, **negative** result is given for other parameter.

The data produced by the instruments can manually be evaluated and reported using their software or can automatically be evaluated and reported using the online ***FastFinder*** software: https://www.ugentec.com/fastfinder. In case the online ***FastFinder*** software is used for the interpretation, the Cq values provided by the software shouldn't be changed and must be reported as they are obtained on the software.

**Table 6. Expected Performance of the Kit Controls**

| Control Type | Control Name | Purpose | Expected Results and Cq Values | |
|---|---|---|---|---|
| | | | ***RNase P* (HEX)** | ***Target* (FAM, HEX, ROX, and CY5)** |
| **Negative Control** | NTC | Contamination control during RT-qPCR | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **Positive Control** | PC | Reagent integrity | **Detected** (Cq≤33) | **Detected** (Cq≤33) |
| **Internal/Extraction Control** | IC | To monitor the integrity of nucleic acid extraction and RT-qPCR from each sample | **Detected** (Cq≤33) If IC Cq>33 check the target Cq | If target Cq≤35, conclude it as IC is valid |

If any control does not perform as described above, the run is considered invalid, and the test is repeated.

1. **Invalid PC (Cq>33 in any channel):** It is recommended to contact the manufacturer, renew the reagents, and repeat the reaction.
2. **Invalid NTC (Cq in any channel):** Repeat the analysis by paying attention to the "**Warnings**" section.
3. **Invalid IC (Cq>33 in HEX channel and no Cq in the other channels):** Repeat the analysis. If the problem continues, then conclude it as an invalid PCR template.

If all the controls are valid, proceed to the interpretation of the results.

**Table 7. Test result examples**

| Target | Internal Control | Report | |
|---|---|---|---|
| Positive (+) | Positive (+) | Report it as **POSITIVE** for the target | 25≤Cq≤35 = Low positive<br>18≤Cq<25 = Positive<br>11≤Cq<18 = High positive<br>Cq<11 = Very high positive |
| Positive (+) | Negative (-) | Report it as **POSITIVE** for the target | |
| Negative (-) | Positive (+) | Report it as **NEGATIVE** for the target | |
| Negative (-) | Negative (-) | **INVALID** Result: Sampling/extraction/inhibition problem<br>Re-extract the specimen and perform testing again. If the result is still invalid, a new specimen should be obtained. If an additional clinical sample is unavailable, report it as **INVALID** | |

**WARNING: On the web page linked with the QR code, examples of the sigmoidal amplification curves are given. The results obtained with this kit should NOT be interpreted without examining these samples.**

## 9. Limitations

- ***Bio-Speedy® Respiratory Tract RT-qPCR MX-24T Panel*** is intended for use by laboratory personnel trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures.
- The clinical specimens shall be collected by a healthcare provider in accordance with the specimen collection guidelines.
- A false-negative result may occur if a specimen is improperly collected, transported, or handled.
- The use of cotton or calcium alginate swabs or swabs with wooden sticks can lead to false-negative results since they may contain substances that inactivate some pathogens and inhibit PCR.
- Mutations within the target regions of the ***Bio-Speedy® Respiratory Tract RT-qPCR MX-24T Panel*** could affect primer and/or probe binding resulting in failure to detect the presence of virus and bacteria.
- Inhibitors or other types of interference may produce a false-negative result. False-negative results may also occur if inadequate numbers of organisms are present in the specimen.

## 10. Explanation of Symbols

| Symbol | Title of Symbol | Symbol | Title of Symbol | Symbol | Title of Symbol |
|---|---|---|---|---|---|
| CE | European Conformity CE Mark | LOT | Batch code | | Keep away from sunlight |
| IVD | *In vitro* diagnostic *medical device* | REF | Catalogue number | | Protect from heat and radioactive sources |
| | Manufacturer | NON STERILE | Non-*sterile* | | Do not use if package is damaged and consult *instructions for use* |
| | Use-by date | | Consult *instructions for use* or consult electronic *instructions for use* | | Keep dry |
| CONTROL - | Negative control | | Caution | | Keep upright |
| CONTROL + | Positive control | | Temperature limit | Σ | Contains sufficient for <*n*> tests |
| CONTROL | Control | | | | |

## 11. Manufacturer and Technical Support

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**Web**: www.bioeksen.com.tr, **e-mail:** info@bioeksen.com.tr,
**Technical Support**: support@bioeksen.com.tr

**Notice to User:** Please inform us about product-related incidents at "**vigilance@bioeksen.com.tr"** within 24 hours.

*For in vitro diagnostic use only.*
*For laboratory professional use only.*

**Cat No:** BS-SY-SI-100/BS-SY-SI-250/BS-SY-SI-500/BS-SY-SI-1000

# COVID-19/Flu RT-qPCR

## Package Insert

### 1. Kit Content

**Table 1: Kit Content**

| Component | Intended Use | Amount (10 µL/Rxn) | | | |
|---|---|---|---|---|---|
| | | 100 Rxns | 250 Rxns | 500 Rxns | 1000 Rxns |
| 2X Prime Script Mix | Optimized ready-to-use mix for RT-qPCR assay | 1 x 500 µL | 1 x 1250 µL | 2 x 1250 µL | 4 x 1250 µL |
| CVD19/FLU Oligo Mix | Specific amplification of the target region in the SARS-CoV-2, Influenza A, Influenza B and Human genome (Internal Control; IC): *ORF1ab*, *N* (FAM), *M* (CY5), *NEP* (ROX), and *RNase P* (HEX) | 1 x 250 µL | 1 x 625 µL | 1 x 1250 µL | 2 x 1250 µL |
| NTC | Negative (No Template) Control (Nuclease-Free Water) | 1 x 1000 µL | 1 x 1000 µL | 1 x 1000 µL | 1 x 1000 µL |
| PC-CVD19/FLU | Positive Control (Synthetic RNA fragment mixture of the targets in the "CVD19/FLU Oligo Mix") | 1 x 250 µL | 1 x 250 µL | 1 x 500 µL | 2 x 500 µL |

**Table 2: Storage Requirements and Shelf Life**

| Component | Transport Condition | Storage Condition | Shelf Life |
|---|---|---|---|
| 2X Prime Script Mix | -22 °C to -18 °C | -22 °C to -18 °C | 12 months |
| CVD19/FLU Oligo Mix | | -22 °C to -18 °C | |
| NTC | | -22 °C to -18 °C / +2 °C to +8 °C | |
| PC-CVD19/FLU | | -22 °C to -18 °C before opening, +2 °C to +8 °C after first thaw | |

**! Each reagent stored at storage temperature, can be used until the expiration date indicated on the tube following the first opening. The expiration date of the kit is determined by the expiration date of the reagents.**

### 2. Materials Required but Not Provided

**Table 3: Components Required but Not Included with The Test**

| Components Required but Not Included with The Test | |
|---|---|
| **1.** Real-Time PCR instrument with FAM, HEX, ROX, and CY5 channels Ramp rate ≥3 °C/sec | **7.** Reaction tubes and their caps/seals compatible with the qPCR instruments and the reaction volume |
| **2.** Adjustable micropipettes and compatible pipette tips (nuclease-free) | **Extra components recommended to use:** |
| **3.** Centrifuge | **8.** Biosafety cabinet for PCR setup |
| **4.** Vortex | **9.** Cold tube rack (for microcentrifuge tubes and PCR tubes/strips) |
| **5.** Nuclease-free water/viral transport medium/serum physiologic | **10.** PPE (Personal Protective Equipment) |
| **6.** 1.5- or 2-mL microcentrifuge tubes (nuclease-free) | |

### 3. Intended Use and Test Principle

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a new, rapidly spreading human beta coronavirus. It was first identified in Wuhan and caused a disease named Coronavirus Disease 2019 (COVID-19). Infection by SARS-CoV-2 causes a respiratory illness that varies in severity from mild upper respiratory symptoms to severe progressive respiratory failure that requires intensive care and can lead to death. The most widely used molecular method approved by the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) to detect SARS-CoV-2 is the real-time reverse transcription polymerase chain reaction (RT-qPCR). Influenza viruses that infect humans are Influenza A (Inf A) and Influenza B (Inf B). Influenza can spread easily to healthy people through droplets from sneezing, coughing, or speaking. Every year, influenza infects many people and even causes deaths. Influenza caused by Inf B virus is not severe and infects mostly children, but influenza caused by Inf A virus is severe and more contagious.

***Bio-Speedy® COVID-19/Flu RT-qPCR*** is a one-step reverse transcription and real-time PCR (RT-qPCR) test intended for the qualitative detection of the RNA from SARS-CoV-2, Influenza A, and Influenza B. The ***Bio-Speedy® COVID-19/Flu RT-qPCR*** is applied to nucleic acids obtained from anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples.

Detection with the kit is achieved via rapid nucleic acid extraction from anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples followed by multiplex RT-qPCR targeting the SARS-CoV-2 specific *Open Reading Frame 1ab* ***(ORF1ab)*** and *Nucleocapsid* ***(N)***, Influenza A specific *Membrane Protein* ***(M)***, and Influenza B specific *Nuclear Export Protein* ***(NEP)*** genes in real-time PCR instruments that are equipped with FAM, HEX, ROX, and CY5 detection channels. **The kit allows to achieve RT-qPCR result in less than 30 minutes (Run time may vary depending on the instrument).**

The oligonucleotide set targeting human ***RNase P*** mRNA functions as a control of the sampling, nucleic acid extraction, reverse transcription, and qPCR since the oligonucleotide set targets the exon-exon junction. The kit also contains negative and positive control templates for testing the contamination and the RT-qPCR reagent stability, respectively.

***Bio-Speedy® COVID-19/Flu RT-qPCR*** is intended for use by laboratory personnel trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures.

### 4. Analytical Specifications

***Bio-Speedy® COVID-19/Flu RT-qPCR*** is validated with ***Bio-Speedy® vNAT® Transfer Tube*** *(Catalog No: BS-NA-513-100),* ***vNAT® Transfer Tube*** *(BS-NA-513m-100),* ***Bio-Speedy® vNAT® Viral Nucleic Acid Buffer*** *(Catalog No: BS-NA-510/BS-NA-510-250/BS-NA-510-500/BS-NA-510-1000),* and ***Bio-Speedy® 5min NA*** *(Catalog No: BS-NA-514-100/BS-NA-514-250/BS-NA-514-500/BS-NA-514-1000)* for nucleic acids prepared from anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples.

The RT-qPCR is carried out in 10 µL reaction volume using the ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** Real-Time PCR systems equipped with the FAM, HEX, ROX, and CY5 detection channel.

***For in vitro diagnostic use only.***
***For laboratory professional use only.***

For the analysis performed on *Bio-Rad Real-Time PCR systems*, the kit has been validated with white reaction tubes specific to these systems. **The clear reaction tubes result in 5-10 times lower fluorescence signal in the *Bio-Rad* instruments compared to the white tubes.** Besides, device-specific reaction tubes should be used on ***BMS*** and ***Applied Biosystems™*** instruments (the specified analytical performance of the kit can only be achieved using the validated tubes).

Limit of Detection (LoD) of the ***Bio-Speedy® COVID-19/Flu RT-qPCR*** is determined as 125 cp/mL for SARS-CoV-2, 125 cp/mL for Influenza A, and 125 cp/mL Influenza B.

The exclusivity of the kit was tested on different pathogens. No cross-reaction was observed in analytical specificity studies performed on reference strains and field isolates. The sensitivity and specificity of the kit were determined as 100.00% and 100.00%, respectively.

## 5. Collection, Storage and Shipment of Clinical Specimens

Clinical samples (anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples) are collected from individuals by a healthcare provider in accordance with the specimen collection guidelines. Anterior nasal swab, nasopharyngeal swab, oropharyngeal swab, and oral/saliva swab samples are transferred into the ***Bio-Speedy® vNAT® Transfer Tube*** (Catalog No: BS-NA-513-100) or ***vNAT® Transfer Tube*** *(Catalog No:BS-NA-513m-100)* containing 2 mL of the ***vNAT® reagent*** or into a sterile transport tube containing 3 mL of Viral Transport Medium (VTM) (Preparation of Viral Transport Medium, Centers for Disease Control and Prevention, SOP#: DSR-052-05). Bronchoalveolar lavage and nasopharyngeal aspirate samples should be transferred into sterile containers containing 3 mL of VTM. Other sample types (saliva, gargle, and sputum samples) should be transferred into preservative-free sterile tubes.

Specimens should be sent to the laboratory within 4 hours after collection at 2 °C to 8 °C. If a delay in delivery for more than 3 days is expected, the samples should be frozen at -70 °C and delivered with dry ice. It is important that the samples should not be exposed to the repeated freeze-thaw.

Specimens in the VTM can be stored at 2 °C to 8 °C for up to 72 hours and specimens in the ***vNAT® Transfer Tube*** can be stored at 2 °C to 8 °C for up to 3 months. If a delay in the RT-qPCR test is expected, specimens can be stored at -70 °C. If not available, specimens can be stored at -20 °C.

## 6. Warnings

1. Specimen processing should be performed in accordance with national biological safety recommendations.
2. Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
3. All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate. Sampling should be carried out by personnel with sufficient knowledge and experience.
4. The kit should be stored away from nucleic acid sources and PCR amplicons.
5. Except for fluid transfers, nucleic acid and positive control tubes should always be kept closed.
6. To prevent contamination of the reaction mixture by previously amplified target sequences, maintain separate work areas, dedicated equipment.
7. Different sets of laboratory coats should be worn pre- and post-PCR.
8. The micropipettes used for pipetting PCR mixes and template nucleic acids should be separate. Filtered and nuclease-free tips should be used.
9. Cotton or calcium alginate swabs or swabs with wooden sticks should not be used since they may contain substances that inactivate some pathogens and inhibit PCR.
10. It is recommended to use swabs with breakable shaft to prevent contamination during sampling.
11. The components in the kit should not be mixed with different lot numbers or chemicals of the same name but from different manufacturers.
12. Master stock reagents should be kept on the cold block during the PCR setup.
13. Kit components should be mixed by gently shaking before use.
14. Maintenance/calibration interval should be determined for all instruments and equipment used with the kit.
15. To avoid false positives due to amplified material, the PCR completed reaction tubes should be disposed of before opening in the laboratory.
16. The wipeable surfaces of the rooms, benches, and devices should be cleaned regularly with freshly diluted 10% bleach solution (0.5% NaClO).
17. Dispose of waste in a designated matter in accordance with local, regional, and federal regulations.

## 7. RT-qPCR Application Protocol

**Table 4: Reaction Set-up and RT-qPCR Program Details**

| Reaction Setup | | RT-qPCR Program | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fast RT-qPCR Protocol | | | | Touchdown RT-qPCR Protocol | | | |
| | | *CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)* and *Mic qPCR (Bio Molecular System - BMS)* | | | | *CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)* and *Mic qPCR (Bio Molecular System - BMS)* | | | |
| **Reagent** | **Volume per Rxn** | **Step** | **Cycle No.** | **Temperature** | **Duration** | **Step** | **Cycle No.** | **Temperature** | **Duration** |
| 2X Prime Script Mix | 5 µL | **Reverse Transcription** | 1 Cycle | 52 °C | 3 min | **Reverse Transcription** | 1 Cycle | 52 °C | 5 min |
| | | **Pre-incubation** | 1 Cycle | 95 °C | 10 sec | **Pre-incubation** | 1 Cycle | 95 °C | 10 sec |
| CVD19/FLU Oligo Mix | 2.5 µL | **Denaturation** | 5 Cycles | 95 °C | 1 sec | **Denaturation** | 12 Touchdown Cycles: 1 °C decrement in annealing temperature per cycle | 95 °C | 1 sec |
| | | **Annealing/Extension** | | 60 °C | 12 sec | **Annealing/Extension** | | 72 °C – 61 °C | 10 sec |
| Template Nucleic Acid | 2.5 µL | **Denaturation** | 35 Cycles | 85 °C | 1 sec | **Denaturation** | 35 Cycles | 85 °C | 1 sec |
| | | **Annealing/Extension** | | 60 °C | 1 sec | **Annealing/Extension** | | 60 °C | 10 sec |
| TOTAL REACTION VOLUME | 10 µL | **Detection (Reading)** | | *FAM/HEX/ROX/CY5* | | **Detection (Reading)** | | *FAM/HEX/ROX/CY5* | |

**WARNING: The RT-qPCR program template of the instrument's software must be downloaded from the manufacturer's website to avoid errors in the manual entry.**

**QR Codes for plate setup for Bio-Rad and thermal profiles for Bio-Rad and Mic.**

**https://www.bioeksen.com.tr/files/covid-19flu/**

## 8. Interpretation of the Assay Results

**For the Fast protocol:**

- The threshold level should be set to 200 RFU for ***CFX96 Touch™/CFX96™ Dx (Bio-Rad)*** **and** ***CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments to calculate Cq values. All other default analysis options in the related software should not be changed for ***CFX96 Touch™/CFX96™ Dx (Bio-Rad)*** **and** ***CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments. For ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)***, "**Non-Assay Green/Parameters/Dynamic**" and "**Auto-Threshold**" options should be selected to calculate Cq values.
- Shape of the amplification curves obtained in the FAM/HEX/ROX/CY5 channels should be examined for all reaction wells returning with Ct values. Ct values should be used in the further interpretation steps if their amplification curve shapes are sigmoidal. **Non-sigmoidal curves should be recorded as negative**. The result is recorded as positive if Ct≤33.
- **For samples with a suspected sigmoidal curve pattern under the threshold in the FAM channel**, Ct-HEX (IC) should be examined. If the Ct-HEX≤30, the sample is reported as negative. If the Ct-HEX>30, the test should be repeated after freezing and thawing the sample. If the problem continues after the freezing and thawing, a new sample is requested.

**For the Touch Down protocol:**

- All default analysis options (e.g. **auto-calculated threshold**) in the related software of ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System – BMS)*** instruments should not be changed to calculate Cq values.
- Shape of the amplification curves obtained in the FAM/HEX/ROX/CY5 channels should be examined for all reaction wells returning with Cq values. All the **sigmoidal curves above the threshold** should be recorded as "**positive**" and their Cq values should be recorded. **Non-sigmoidal curves** should be recorded as "**negative**".
- For samples with a sigmoidal curve below the auto-calculated threshold for the ***CFX96 Touch™/CFX96™ Dx (Bio-Rad) and CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments, **the threshold level should be manually set to 200 RFU. If the sigmoidal curve exceeds the threshold, the Ct value should be recorded as "35"** and the sample should be reported as **positive**. For samples with a sigmoidal curve below the auto-calculated threshold for ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** should be reported as **positive** and **their Ct values should be recorded as "35"**.

**Table 5. Expected Performance of the Kit Controls**

| Control Type | Control Name | Purpose | Expected Results and Cq Values | |
|---|---|---|---|---|
| | | | *Internal Control* | *Target* |
| **Negative Control** | NTC | Contamination control during RT-qPCR | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **No template addition** | NRC | Reagent contamination control | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **Positive Control** | PC | Reagent integrity | **Detected** (Cq≤33) | **Detected** (Cq≤33) |
| **Internal/Extraction Control** | IC | To monitor the integrity of nucleic acid extraction and qPCR from each sample | **Detected** (Cq≤33)<br>If IC Cq>33 check the target Cq | For the Touchdown Protocol;<br>If target Ct≤35.0, conclude it as IC is valid<br>For the Fast Protocol;<br>If target Ct≤33.0, conclude it as IC is valid |

If any control does not perform as described above, the run is considered invalid, and the test is repeated.

1. **Invalid PC (Cq>33 in any channel):** It is recommended to contact the manufacturer, renew the reagents, and repeat the reaction.
2. **Invalid NTC (No Cq in any channel):** Repeat the analysis by paying attention to the "**Warnings**" section.
3. **Invalid NRC (No Cq in any channel):** Contact the manufacturer, renew the reagents, and repeat the reaction.
4. **Invalid IC (Cq>33 in HEX channel and no Cq in the other channels):** Repeat the analysis. If the problem continues, then conclude it as an invalid PCR template.

If all the controls are valid, proceed to the interpretation of the results.

**Table 6. Interpretation of Patient Samples**

| Target | Internal Control | Results Interpretation | Action |
|---|---|---|---|
| **Positive (+)** | **Positive (+)** | Results are VALID,<br>Target RNA is detected | Report it as **POSITIVE** |
| **Positive (+)** | **Negative (-)** | Results are VALID,<br>Target RNA is detected | Report it as **POSITIVE** |
| **Negative (-)** | **Positive (+)** | Results are VALID,<br>Target RNA is not detected | Report it as **NEGATIVE** |
| **Negative (-)** | **Negative (-)** | Results are INVALID<br>(sampling/extraction/inhibition problem) | Re-extract the specimen and perform testing again. If the result is still invalid, a new specimen should be obtained. If an additional clinical sample is unavailable, report it as **INVALID** |

**WARNING: On the web page linked with the QR code, examples of the sigmoidal amplification curves are given. The results obtained with this kit should NOT be interpreted without examining these samples.**

## 9. Limitations

- ***Bio-Speedy® COVID-19/Flu RT-qPCR*** is intended for use by laboratory personnel trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures.
- The clinical specimens shall be collected by a healthcare provider in accordance with the specimen collection guidelines.
- A false-negative result may occur if a specimen is improperly collected, transported, or handled.
- The use of cotton or calcium alginate swabs or swabs with wooden sticks can lead to false-negative results since they may contain substances that inactivate some pathogens and inhibit PCR.
- Mutations within the target regions of the ***Bio-Speedy® COVID-19/Flu RT-qPCR*** could affect primer and/or probe binding resulting in failure to detect the presence of agents.
- Inhibitors or other types of interference may produce a false-negative result. False-negative results may also occur if inadequate numbers of organisms are present in the specimen.

## 10. Explanation of Symbol

| Symbol | Title of Symbol | Symbol | Title of Symbol | Symbol | Title of Symbol |
|---|---|---|---|---|---|
| CE | European Conformity CE Mark | LOT | Batch code | Σ | Contains sufficient for *<n>* tests |
| IVD | *In vitro* diagnostic *medical device* | REF | Catalogue number | | Keep away from sunlight |
| | Manufacturer | NON STERILE | Non-*sterile* | | Protect from heat and radioactive sources |
| | Use-by date | | Consult *instructions for use* or consult electronic *instructions for use* | | Do not use if package is damaged and consult *instructions for use* |
| CONTROL - | Negative control | | Caution | | Keep dry |
| CONTROL + | Positive control | | Temperature limit | | Keep it upright |
| CONTROL | Control | | | | |

## 11. Manufacturer and Technical Support

**Bioeksen R&D Technologies Incorporated Company**
**Address:** Resitpasa Mh. Katar Cd., 4/B-105. 34467, Sariyer, Istanbul, TURKEY.
**Phone:** +90 (212) 285 10 17, **Fax:** +90 (212) 285 10 18
**Web**: www.bioeksen.com.tr, E-**mail:** info@bioeksen.com.tr,
**Technical Support**: support@bioeksen.com.tr

**Notice to User:** Please inform us about product-related incidents at "**vigilance@bioeksen.com.tr**" within 24 hours.

*For in vitro diagnostic use only.*
*For laboratory professional use only.*

**Cat No:** CCHFVD01100

# CCHFV RT-qPCR Detection Kit

## Package Insert

### 1. Kit Content

**Table 1. Kit Content**

| Component | Intended Use | Amount (10 µL/Rxn) |
|---|---|---|
| | | 100 Rxns |
| 2X Prime Script Mix | Optimized ready-to-use mix for RT-qPCR assay | 1 x 500 µL |
| CCHF Oligo Mix | Specific nucleic acid amplification and detection:<br>FAM: Nairovirus specific *N* gene<br>HEX: Human genome *RNase P* as an internal control | 1 x 250 µL |
| NTC | Negative (No Template) Control<br>(Nuclease-Free Water) | 1 x 1000 µL |
| PC-CCHF | Positive Control (Synthetic RNA fragment mixture of the targets in the "CCHF Oligo Mix") | 1 x 250 µL |

**Table 2. Storage Requirements and Shelf Life**

| Component | Transport Conditions | Storage Conditions | Shelf Life |
|---|---|---|---|
| 2X Prime Script Mix | -22 °C to +8 °C | -22 °C to -18 °C | 12 months |
| CCHF Oligo Mix | | -22 °C to -18 °C | |
| NTC | | -22 °C to -18 °C / +2 °C to +8 °C | |
| PC-CCHF | | -22 °C to -18 °C before opening, +2 °C to +8 °C after first thaw | |

**! Each reagent stored at storage temperature can be used until the expiration date indicated on the tube following the first opening. The expiration date of the kit is determined by the expiration date of the reagents.**

### 2. Materials Required but Not Provided

**Table 3. Components Required but not Included with the Test**

| Components Required but Not Included with The Test | |
|---|---|
| **1.** Real-Time PCR instrument with FAM and HEX channels Ramp rate ≥3 °C/sec | **7.** Reaction tubes and their caps/seals compatible with the qPCR instruments and the reaction volume |
| **2.** Adjustable micropipettes and compatible pipette tips (nuclease-free) | **Extra components recommended to use:** |
| **3.** Centrifuge | **8.** Biosafety cabinet for PCR setup |
| **4.** Vortex | **9.** Cold tube rack (for microcentrifuge tubes and PCR tubes/strips) |
| **5.** Nuclease-free water/viral transport medium/serum physiologic | **10.** PPE (Personal Protective Equipment) |
| **6.** 1.5- or 2-mL microcentrifuge tubes (nuclease-free) | |

### 3. Intended Use and Test Principle

Crimean Congo Hemorrhagic Fever (CCHF) is a fatal disease with a mortality rate of 5-30% caused by an RNA virus identified in the genus Nairovirus from the order Bunyaviridea and the family Nairoviridae. The virus was first observed in the Western Crimean steppes in the summer of 1944 and 1945. The disease is often observed in Africa, Western Asia, the Middle East, and Eastern Europe. Crimean Congo Hemorrhagic Fever Virus outbreaks were reported in Bulgaria, Macedonia, Pakistan, Iraq, Afghanistan, Iran, Kosovo, Kazakhstan, sub-Saharan African countries, the former Soviet Union, Yugoslavia, Greece, Arabia, Dubai, Kuwait, Mauritania, and China. The disease was first seen in Turkey in the spring and summer of 2002, and as a result of the studies conducted by the Ministry of Health of the Republic of Turkey, it has been confirmed that the disease is CCHF. As a result of genetic analyzes applied in the following years, It has been shown that viral nucleic acids obtained from patients and ticks reported in Turkey show close similarities with those from Kosovo, Albania, Greece, Bulgaria, and Russia.

***Bio-Speedy® CCHFV RT-qPCR Detection Kit*** is a one-step reverse transcription and real-time PCR (RT-qPCR) test intended for the qualitative detection of the RNA from Nairovirus. The ***Bio-Speedy® CCHFV RT-qPCR Detection Kit*** is applied to nucleic acids obtained from serum, plasma, and whole blood samples. The kit is non-automated and functions as an aid to diagnosis.

Detection with the kit is achieved via rapid nucleic acid extraction from serum, plasma, and whole blood samples followed by multiplex RT-qPCR targeting the Nairovirus specific *Nucleocapsid* (***N***) gene in real-time PCR instruments that are equipped with FAM and HEX detection channels. **The kit allows to achieve RT-qPCR result in 43 minutes (Run time may vary depending on the instrument)**.

The oligonucleotide set targeting human ***RNase P*** mRNA functions as a control of the sampling, nucleic acid extraction, reverse transcription, and qPCR since the oligonucleotide set targets the exon-exon junction. The kit also contains negative and positive control templates for testing the contamination and the RT-qPCR reagent stability, respectively.

***Bio-Speedy® CCHFV RT-qPCR Detection Kit*** is intended for use by laboratory personnel trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures.

### 4. Analytical Specifications

***Bio-Speedy® CCHFV RT-qPCR Detection Kit*** is validated with a robotic extraction system such as ***Zybio EXM3000 Nucleic Acid Isolation System*** *(Model No: EXM3000)* for nucleic acids prepared from serum, plasma, and whole blood samples.

The RT-qPCR is carried out in 10 µL reaction volume using the ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** Real-Time PCR systems equipped with the FAM and HEX detection channels.

For the analysis performed on *Bio-Rad Real-Time PCR systems*, the kit has been validated with white reaction tubes specific to these systems. **The clear reaction tubes result in 5-10 times lower fluorescence signal in the *Bio-Rad* instruments compared to the white tubes.** Besides, device-specific reaction tubes should be used on ***BMS*** instruments (the specified analytical performance of the kit can only be achieved using the validated tubes).

Limit of Detection (LoD) of the ***Bio-Speedy® CCHFV RT-qPCR Detection Kit*** is determined as 1000 cp/mL for the Nairovirus for serum, plasma, and whole blood samples extracted using the ***Zybio EXM3000 Nucleic Acid Isolation System***.

The exclusivity of the kit was tested on different pathogens. No cross-reaction was observed in analytical specificity studies performed on reference strains and field isolates. The sensitivity and specificity of the kit were determined as 99.67% and 100.00%, respectively.

## 5. Collection, Storage, and Shipment of Clinical Specimens

Collect whole blood into commercially available anticoagulant-treated tubes, e.g. EDTA-treated (lavender tops) or citrate-treated (light blue tops) for whole blood samples. Whole blood samples in tubes are preferably stored at 2 °C to 8 °C and transferred to the laboratory within 24 hours at the latest. For long-term storage, samples should be stored at -20°C. Following the centrifugation of serum or plasma samples, it is crucial to immediately transfer the serum or plasma samples using a Pasteur pipette into a sterile polypropylene tube. During handling, the samples should be kept between 2 and 8 °C. The serum or plasma samples should be portioned into 0.5 ml aliquots, stored, and transported at -20°C or lower if they won't be analyzed immediately. It is important to avoid freeze-thaw cycles.

## 6. Preparation of Nucleic Acid Samples

The automated ***Zybio EXM3000 Nucleic Acid Isolation System*** extraction is used according to the manufacturer's instructions.

## 7. Warnings

1. Specimen processing should be performed in accordance with national biological safety recommendations.
2. Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
3. All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate. Sampling should be carried out by personnel with sufficient knowledge and experience.
4. The kit should be stored away from nucleic acid sources and PCR amplicons.
5. Except for fluid transfers, nucleic acid, and positive control tubes should always be kept closed.
6. To prevent contamination of the reaction mixture by previously amplified target sequences, maintain separate work areas, and dedicated equipment.
7. Different sets of laboratory coats should be worn pre- and post-PCR.
8. The micropipettes used for pipetting PCR mixes and template nucleic acids should be separate. Filtered and nuclease-free tips should be used.
9. The components in the kit should not be mixed with different lot numbers or chemicals of the same name but from different manufacturers.
10. Master stock reagents should be kept on the cold block during the PCR setup.
11. Kit components should be mixed by gently shaking before use.
12. Maintenance/calibration interval should be determined for all instruments and equipment used with the kit.
13. To avoid false positives due to amplified material, the PCR-completed reaction tubes should be disposed of before opening in the laboratory.
14. The wipeable surfaces of the rooms, benches and devices should be cleaned regularly with freshly diluted 10% bleach solution (0.5% NaClO).
15. Dispose of waste in a designated matter in accordance with local, regional, and federal regulations.

## 8. RT-qPCR Application Protocol

**Table 4. Reaction Set-up and RT-qPCR Program Details**

| Reaction Setup | | RT-qPCR Program | | | | QR Code for Thermal Protocol |
|---|---|---|---|---|---|---|
| | | *CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad) and Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)* | | | | |
| **Reagent** | **Volume/Rxn** | **Step** | **Cycle No.** | **Temperature** | **Duration** | |
| 2X Prime Script Mix | 5 µL | **Reverse Transcription** | 1 Cycle | 52 °C | 3 min | |
| | | **Pre-Incubation** | 1 Cycle | 95 °C | 10 sec | |
| CCHF Oligo Mix | 2.5 µL | **Denaturation** | 12 Touch Down Cycles: 1 °C decrement in annealing temperature per cycle | 95 °C | 1 sec | |
| | | **Annealing and Extension** | | 67 °C – 56 °C | 10 sec | |
| Template Nucleic Acid | 2.5 µL | **Denaturation** | 35 Cycles | 85 °C | 1 sec | |
| | | **Annealing and Extension** | | 55 °C | 10 sec | |
| **Total Reaction Volume** | **10 µL** | **Detection (Reading)** | | (FAM-Green)/(HEX-Yellow) | | https://www.bioeksen.com.tr/files/cchfv |

**WARNING: The RT-qPCR program template of the instrument's software must be downloaded from the manufacturer's website to avoid errors in the manual entry.**

## 9. Interpretation of the Assay Results

- All default analysis options (e.g. **auto-calculated threshold**) in the related software of ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System – BMS)*** instruments should not be changed to calculate Cq values.
- Shape of the amplification curves obtained in the **FAM**/**HEX** channels should be examined for all reaction wells returning with Cq values. All the **sigmoidal curves above the threshold** should be recorded as "**positive,**" and their Cq values should be recorded. **Non-sigmoidal curves** should be recorded as "**negative**."
- For samples with a sigmoidal curve below the auto-calculated threshold for the ***CFX96 Touch™/CFX96™ Dx (Bio-Rad) and CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments, **the threshold level should be manually set to 200 RFU. If the sigmoidal curve exceeds the threshold, the Cq value should be recorded as "35,"** and the sample should be reported as "**positive**".
- For **samples with a sigmoidal curve below the auto-calculated threshold** for ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** should be reported as "**positive,**" and **their Cq values should be recorded as "35"**.

**Table 5. Expected Performance of the Kit Controls**

| Control Type | Control Name | Purpose | Expected Results and Cq Values | |
|---|---|---|---|---|
| | | | ***Internal Control* (HEX)** | ***Target* (FAM)** |
| **Negative Control** | NTC | Contamination control during RT-qPCR | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **No template addition** | NRC | Reagent contamination control | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **Positive Control** | PC | Reagent integrity | **Detected** (Cq≤33) | **Detected** (Cq≤33) |
| **Internal/Extraction Control** | IC | To monitor the integrity of nucleic acid extraction and RT-qPCR from each sample | **Detected** (Cq≤33)<br>If IC Cq>33 check the target Cq | If target Cq≤35.0, conclude it as IC is valid |

If any control does not perform as described above, the run is considered invalid, and the test is repeated.

1. **Invalid PC (Cq>33 in any channel):** It is recommended to contact the manufacturer, renew the reagents, and repeat the reaction.
2. **Invalid NTC (No Cq in any channel):** Repeat the analysis by paying attention to the "**Warnings**" section.
3. **Invalid NRC (No Cq in any channel):** Contact the manufacturer, renew the reagents, and repeat the reaction.
4. **Invalid IC (Cq>33 in HEX channel and no Cq in the other channels):** Repeat the analysis. If the problem continues, then conclude it as an invalid PCR template.

If all the controls are valid, proceed to the interpretation of the results.

**Table 6. Interpretation of Patient Samples**

| Target | Internal Control | Results Interpretation | Action |
|---|---|---|---|
| **Positive (+)** | **Positive (+)** | Results are VALID,<br>Target RNA is detected | Report it as **POSITIVE** |
| **Positive (+)** | **Negative (-)** | Results are VALID,<br>Target RNA is detected | Report it as **POSITIVE** |
| **Negative (-)** | **Positive (+)** | Results are VALID,<br>Target RNA is not detected | Report it as **NEGATIVE** |
| **Negative (-)** | **Negative (-)** | Results are INVALID<br>(sampling/extraction/inhibition problem) | Re-extract the specimen and perform testing again. If the result is still invalid, a new specimen should be obtained. If an additional clinical sample is unavailable, report it as **INVALID** |

**WARNING: On the web page linked with the QR code, examples of the sigmoidal amplification curves are given. The results obtained with this kit should NOT be interpreted without examining these samples.**

## 10. Limitations

- ***Bio-Speedy® CCHFV RT-qPCR Detection Kit*** is intended for use by laboratory personnel trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures.
- The clinical specimens shall be collected by a healthcare provider in accordance with the specimen collection guidelines.
- A false-negative result may occur if a specimen is improperly collected, transported, or handled.
- Mutations within the target regions of the ***Bio-Speedy® CCHFV RT-qPCR Detection Kit*** could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Inhibitors or other types of interference may produce a false-negative result. False-negative results may also occur if inadequate numbers of organisms are present in the specimen.

## 11. Explanation of Symbol

| Symbol | Title of Symbol | Symbol | Title of Symbol | Symbol | Title of Symbol |
|---|---|---|---|---|---|
| CE | European Conformity CE Mark | LOT | Batch code | Σ | Contains sufficient for <*n*> tests |
| IVD | *In vitro* diagnostic *medical device* | REF | Catalogue number | | Keep away from sunlight |
| | Manufacturer | NON STERILE | Non-*sterile* | | Protect from heat and radioactive sources |
| | Use-by date | | Consult *instructions for use* or consult electronic *instructions for use* | | Do not use if package is damaged and consult *instructions for use* |
| CONTROL - | Negative control | | Caution | | Keep dry |
| CONTROL + | Positive control | | Temperature limit | | Keep it upright |
| CONTROL | Control | | | | |

## 12. Manufacturer and Technical Support

**Bioeksen R&D Technologies Incorporated Company**
**Address:** Resitpasa Mh. Katar Cd., 4/B-105. 34467, Sariyer, Istanbul, TURKEY.
**Phone:** +90 (212) 285 10 17, **Fax:** +90 (212) 285 10 18
**Web**: www.bioeksen.com.tr, **E-mail:** info@bioeksen.com.tr,
**Technical Support**: support@bioeksen.com.tr

**Notice to User:** Please inform us about product-related incidents at "**vigilance@bioeksen.com.tr**" within 24 hours.

*For in vitro diagnostic use only.*
*For laboratory professional use only.*

**Cat No:** BS-MEV-DTC-320-100

# Measles Virus Real-Time PCR Detection Kit

## Package Insert

### 1. Kit Content

**Table 1: Kit Content**

| Component | Description of the Components | Quantity (10 µL/Rxn) |
|---|---|---|
| | | 100 Rxns |
| 2X Prime Script Mix | Optimized ready-to-use mix for RT-qPCR assay | 1 x 500 µL |
| MeV Oligo Mix | Specific amplification of the target region in the Measles virus and Human genome (Internal Control; IC): *N* (FAM) and *RNase P* (HEX) | 1 x 250 µL |
| NTC | Negative (No Template) Control (Nuclease-Free Water) | 1 x 1000 µL |
| PC-MeV | Positive Control (Synthetic RNA fragment mixture of the targets in the "MeV Oligo Mix") | 1 x 250 µL |

**Table 2: Storage Requirements and Shelf Life**

| Component | Transport Conditions | Storage Conditions | Shelf Life |
|---|---|---|---|
| 2X Prime Script Mix | -22 -18 °C | -22 -18 °C | 12 months |
| MeV Oligo Mix | | -22 -18 °C | |
| NTC | | -22 -18 °C/2-8 °C | |
| PC-MeV | | -22 -18 °C before opening, 2-8 °C after first thaw | |

**Each reagent stored at storage temperature, can be used until the expiration date indicated on the tube following the first opening. The expiration date of the kit is determined by the expiration date of the reagents.**

### 2. Materials Required but Not Provided

**Table 3: Components Required but not Included with the Test**

| Components Required but not Included with the Test | |
|---|---|
| **1.** Real-Time PCR instrument with FAM and HEX channels, Ramp rate ≥3 °C/sec | **7.** Reaction tubes and their caps/seals compatible with the qPCR instruments and the reaction volume |
| **2.** Adjustable micropipettes and compatible pipet tips (nuclease-free) | **Extra components recommended to use:** |
| **3.** Centrifuge | **8.** Biosafety cabinet for PCR setup |
| **4.** Vortex | **9.** Cold tube rack (for microcentrifuge tubes and PCR tubes/strips) |
| **5.** Nuclease-free water/viral transport medium/serum physiologic | **10.** PPE (Personal Protective Equipment) |
| **6.** 1.5- or 2-mL microcentrifuge tubes (nuclease-free) | |

### 3. Intended Use and Test Principle

Measles is an acute viral respiratory illness, caused by a single-stranded, enveloped RNA virus. It is one of the most contagious of all infectious diseases; up to 9 out of 10 susceptible persons with close contact to a measles patient will develop measles. The virus is transmitted by direct contact with infectious droplets or by airborne spread when an infected person breathes, coughs, or sneezes. It is characterized by a prodrome of fever and malaise, cough, coryza, and conjunctivitis. The rash usually appears about 14 days after a person is exposed. The rash spreads from the head to the trunk to the lower extremities. Patients are considered to be contagious from 4 days before to 4 days after the rash appears.

***Bio-Speedy® Measles Virus Real-Time PCR Detection Kit*** is a one-step reverse transcription and real-time PCR (RT-qPCR) test intended for the qualitative detection of the RNA from Measles virus. The ***Bio-Speedy® Measles Virus Real-Time PCR Detection Kit*** is applied to nucleic acid isolates obtained from nasopharyngeal swab, oropharyngeal swab, and urine samples.

Detection with the kit is achieved via rapid nucleic acid extraction from nasopharyngeal swab, oropharyngeal swab, and urine samples followed by multiplex RT-qPCR targeting the Measles virus specific *Nucleocapsid* ***(N)*** gene in real-time PCR instruments that are equipped with FAM and HEX detection channels. **The kit allows to achieve RT-qPCR result in 30 minutes (Run time may vary depending on the instrument).**

The oligonucleotide set targeting human ***RNase P*** mRNA functions as a control of the sampling, nucleic acid extraction, reverse transcription, and qPCR since the oligonucleotide set targets the exon-exon junction. The kit also contains negative and positive control templates for testing the contamination and the qPCR reactive stability, respectively.

***Bio-Speedy® Measles Virus Real-Time PCR Detection Kit*** is intended for use by laboratory personnel trained in the techniques of qPCR and *in vitro* diagnostic procedures.

### 4. Analytical Specifications

***Bio-Speedy® Measles Virus Real-Time PCR Detection Kit*** is validated with ***vNAT® Extraction Consumables*** *(****vNAT® Transfer Tube*** *(Catalog No: BS-NA-513-100 and BS-NA-513m-100),* ***vNAT® Viral Nucleic Acid Buffer*** *(Catalog No: BS-NA-510))* and Robotic Extraction systems such as ***Zybio EXM3000 Nucleic Acid Isolation System*** *(Robot Catalog No: ZBI-EXM3000)* for nucleic acids extracted from nasopharyngeal swab, oropharyngeal swab, and urine samples.

The RT-qPCR is carried out in 10 µL reaction volume using the ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad), QuantStudio™ 5, 0.1 mL/QuantStudio™ 5 Dx, 0.1 mL (Applied Biosystems™),*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** Real-Time PCR systems equipped with the FAM and HEX detection channel. ***Bio-Speedy® Measles Virus Real-Time PCR Detection Kit*** is also validated with the ***Hamilton Microlab® STAR Line***, ***Hamilton Microlab® NIMBUS Line***, ***Hamilton Microlab® VANTAGE Line***, ***Biomek i-Series (Beckman Coulter)***, and ***Tecan Robotics*** liquid handler systems.

The data produced by the instruments can manually be evaluated and reported using their software or can automatically be evaluated and reported using the online ***FastFinder*** software: https://www.ugentec.com/fastfinder.

For the analysis performed on *Bio-Rad Real-Time PCR systems*, the kit has been validated with white reaction tubes specific to these systems. **The clear reaction tubes result in 5-10 times lower fluorescence signal in the *Bio-Rad* instruments compared to the white tubes.** Besides, device-specific reaction tubes should be used on ***BMS*** and ***Applied Biosystems™*** instruments (the specified analytical performance of the kit can only be achieved using the validated tubes).

Limit of Detection (LoD) of the ***Bio-Speedy® Measles Virus Real-Time PCR Detection Kit*** is determined as 1000 cp/mL.

The exclusivity of the kit was tested on different pathogens. No cross reaction was observed in analytical specificity studies performed on reference strains and field isolates. The sensitivity and specificity of the kit were determined as 100.00% and 100.00%, respectively.

## 5. Collection, Storage and Shipment of Clinical Specimens

Clinical samples (nasopharyngeal swab, oropharyngeal swab, and urine samples) are collected from individuals by a healthcare provider in accordance with the specimen collection guidelines. Nasopharyngeal swab and oropharyngeal swab samples are transferred into the ***vNAT® Transfer Tube*** (*Catalog No: BS-NA-513-100 and BS-NA-513m-100*) containing 2 mL of the ***vNAT® reagent*** or into a sterile transport tube containing 3 mL of Viral Transport Medium (VTM) (Preparation of Viral Transport Medium, Centers for Disease Control and Prevention, SOP#: DSR-052-05). Urine should be transferred into preservative-free sterile tubes.

Specimens should be sent to the laboratory within 2h to 4h after collection for testing. If the shipping time is likely to be more than 4 h, specimens should be immediately placed on refrigerant gel packs or at 2 °C to 8 °C (refrigeration) for transport to the testing laboratory. If delivery will be delayed for more than 3d to 4d, the specimen should be frozen at −70 °C. If not available, specimens can be stored at −20 °C. During specimen transportation, repeated freezing and thawing should be avoided as far as possible to prevent degradation of nucleic acid.

Specimens in the VTM can be stored at 2-8 °C for up to 72 hours and specimens in the ***vNAT® Transfer Tube*** can be stored at 2-8 °C for up to 3 months. If a delay in the qPCR test is expected, specimens can be stored at -70 °C. If not available, specimens can be stored at −20 °C.

## 6. Warnings

1. Specimen processing should be performed in accordance with national biological safety recommendations.
2. Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
3. All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate. Sampling should be carried out by personnel with sufficient knowledge and experience.
4. The kit should be stored away from nucleic acid sources and PCR amplicons.
5. Except for fluid transfers, nucleic acid and positive control tubes should always be kept closed.
6. To prevent contamination of the reaction mixture by previously amplified target sequences, maintain separate work areas, dedicated equipment.
7. Different sets of laboratory coats should be worn pre- and post-PCR.
8. The micropipettes used for pipetting PCR mixes and template nucleic acids should be separate. Filtered and nuclease-free tips should be used.
9. Cotton or calcium alginate swabs or swabs with wooden sticks should not be used since they may contain substances that inactivate some pathogens and inhibit PCR.
10. It is recommended to use swabs with breakable shaft to prevent contamination during sampling.
11. The components in the kit should not be mixed with different lot numbers or chemicals of the same name but from different manufacturers.
12. Master stock reagents should be kept on the cold block during the PCR setup.
13. Kit components should be mixed by gently shaking before use.
14. Maintenance/calibration interval should be determined for all instruments and equipment used with the kit.
15. To avoid false positives due to amplified material, the PCR completed reaction tubes should be disposed of before opening in the laboratory.
16. The wipeable surfaces of the rooms, benches, and devices should be cleaned regularly with freshly diluted 10% bleach solution (0.5% NaClO).
17. Dispose of waste in a designated matter in accordance with local, regional, and federal regulations.

## 7. RT-qPCR Application Protocol

Before starting the assay, please consider the following:

1. The kit was validated only for the template nucleic acid volume that is 25% of the total qPCR volume.
2. The kit cannot be used with real-time PCR instruments without the periodic maintenance records.
3. For ***QuantStudio™ 5, 0.1 mL/QuantStudio™ 5 Dx, 0.1 mL (Applied Biosystems™)*** instruments **"Passive Reference Dye"** should be **"None"** selected.
4. **It is recommended to use validated qPCR plate/strip with the kit!** The specified analytical performance of the kit can only be achieved using the validated tubes.
5. **For testing the contamination, setup two different negative control reactions with and without addition of NTC.**

Program the qPCR device as follows and add the reagents to the qPCR tubes, close the tubes, place them into the qPCR instrument and start the run (Table 4).

**Table 4: Reaction Set-up and RT-qPCR Program Details**

| Reaction Setup | | RT-qPCR Program | | | |
|---|---|---|---|---|---|
| | | *CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad), Mic qPCR (Bio Molecular System - BMS), QuantStudio™ 5, 0.1 mL/QuantStudio™ 5 Dx, 0.1 mL (Applied Biosystems™)* | | | |
| **Reagent** | **Volume/Rxn** | **Step** | **Cycle No.** | **Temperature** | **Duration** |
| 2X Prime Script Mix | 5 µL | **Reverse Transcription** | 1 | 52 °C | 5 min |
| MeV Oligo Mix | 2.5 µL | **Hold** | 1 | 95 °C | 10 sec |
| Template Nucleic Acid | 2.5 µL | **Denaturation** | 40 | 95 °C | 1 sec |
| | | **Annealing/Extension** | | 55 °C | 5 sec |
| TOTAL REACTION VOLUME | 10 µL | **Detection (Reading)** | **FAM/HEX** | | |

## 8. Interpretation of the Assay Results

- The threshold level should be set to 200 RFU for ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments to calculate Cq values. All other default analysis options in the related software should not be changed for ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments. For ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)***, **"Non-Assay Green/Parameters/Dynamic"** and **"Auto-Threshold"** options should be selected to calculate Cq values. **"Auto-Threshold"** options should be selected to calculate Cq values for ***QuantStudio™ 5, 0.1 mL/QuantStudio™ 5 Dx, 0.1 mL (Applied Biosystems™)*** instruments.
- Shape of the amplification curves obtained in the **FAM**/**HEX** channels should be examined for all reaction wells returning with Cq values. Cq values should be used in the further interpretation steps if their amplification curve shapes are sigmoidal. **Non-sigmoidal curves should be recorded as negative.** The result is recorded as positive if Cq≤38.
- **For samples with a suspected sigmoidal curve pattern under the threshold in the targets' channel, Cq value of the IC should be examined. If the IC Cq≤34, the sample is reported as negative. If the Cq>34, the test should be repeated after freezing and thawing the sample. If the problem continues after the freezing and thawing, a new sample is requested.**

**Table 5: Expected Performance of the Kit Controls**

| Control Type | Control Name | Purpose | Expected Results and Cq Values | |
|---|---|---|---|---|
| | | | RNase P (HEX) | N (FAM) |
| **Negative Control** | NTC | Contamination control during RT-qPCR | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **No template addition** | NRC | Reactive contamination control | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **Positive Control** | PC | Reagent integrity | **Detected** (Cq≤38) | **Detected** (Cq≤38) |
| **Internal/Extraction Control** | IC | To monitor the integrity of nucleic acid extraction and qPCR from each sample | **Detected** (Cq≤38)<br>If IC Cq>38.0 check the target Cq | If target Cq≤38.0, conclude as IC is valid |

If any control does not perform as described above, the run is considered invalid, and the test is repeated.

1. **Invalid PC (Cq>38 in any channel):** It is recommended to contact the manufacturer, renew the reagents, and repeat the reaction.
2. **Invalid NTC (Cq≤38 in any channel):** Repeat the analysis by paying attention to the "**Warnings**" section.
3. **Invalid NRC (Cq≤38 in any channel):** Contact the manufacturer, renew the reagents, and repeat the reaction.
4. **Invalid IC (Cq>38 in HEX channel):** Repeat the analysis. If the problem continues, then conclude as an invalid PCR template.

If all the controls are valid, proceed to the interpretation of the results.

**Table 6: Interpretation of Patient Samples**

| N (FAM) (Positive for Cq≤38) | RNase P (HEX) (Positive for Cq≤38) | Results Interpretation | Action |
|---|---|---|---|
| **Positive (+)** | **Positive (+)** | Results are VALID,<br>Measles virus RNA is detected | Report as **POSITIVE** |
| **Positive (+)** | **Negative (-)** | Results are VALID,<br>Measles virus RNA is detected | Report as **POSITIVE** |
| **Negative (-)** | **Positive (+)** | Results are VALID,<br>Measles virus RNA is not detected | Report as **NEGATIVE** |
| **Negative (-)** | **Negative (-)** | Results are INVALID<br>(sampling/extraction/inhibition problem) | Re-extract the specimen and perform testing again. If the result is still invalid, a new specimen should be obtained. If additional clinical sampleis unavailable, report as **INVALID** |

**WARNING: On the web page linked with the QR code, examples of the sigmoidal amplification curves are given. The results obtained with this kit should NOT be interpreted without examining these samples.**

## 9. Limitations

- ***Bio-Speedy® Measles Virus Real-Time PCR Detection Kit*** is intended for use by laboratory personnel trained in the techniques of qPCR and *in vitro* diagnostic procedures.
- The clinical specimens shall be collected by a healthcare provider in accordance with the specimen collection guidelines.
- A false negative result may occur if a specimen is improperly collected, transported, or handled.
- The use of cotton or calcium alginate swabs or swabs with wooden sticks can lead to false negative results since they may contain substances that inactivate some pathogens and inhibit PCR.
- Mutations within the target regions of the ***Bio-Speedy® Measles Virus Real-Time PCR Detection Kit*** could affect primer and/or probe binding resulting in failure to detect the presence of agents.
- Inhibitors or other types of interference may produce a false negative result. False negative results may also occur if inadequate numbers of organisms are present in the specimen.

## 10. Explanation of Symbol

| Symbol | Title of Symbol | Symbol | Title of Symbol | Symbol | Title of Symbol |
|---|---|---|---|---|---|
| CE | European Conformity CE Mark | LOT | Batch code | Σ | Contains sufficient for *<n>* tests |
| IVD | *In vitro* diagnostic *medical device* | REF | Catalogue number | | Keep away from sunlight |
| | Manufacturer | NON STERILE | Non-*sterile* | | Protect from heat and radioactive sources |
| | Use-by date | | Consult *instructions for use* or consult electronic *instructions for use* | | Do not use if package is damaged and consult *instructions for use* |
| CONTROL - | Negative control | | Caution | | Keep dry |
| CONTROL + | Positive control | | Temperature limit | | Keep it upright |
| CONTROL | Control | | | | |

## 11. Manufacturer and Technical Support

**Bioeksen R&D Technologies Incorporated Company**
**Address:** Resitpasa Mh. Katar Cd., 4/B-105. 34467, Sariyer, Istanbul, TURKEY.
**Phone:** +90 (212) 285 10 17, **Fax:** +90 (212) 285 10 18
**Web**: www.bioeksen.com.tr, **e-mail:** info@bioeksen.com.tr,
**Technical Support**: support@bioeksen.com.tr

**Notice to User:** Please inform us about product-related incidents at "**vigilance@bioeksen.com.tr**" within 24 hours.

*For in vitro diagnostic use only.*
*For laboratory professional use only.*

**Cat No:** BS-ME-HNS-100

# H. influenzae/N. meningitidis/S. pneumoniae qPCR Kit

## Package Insert

## 1. Kit Content

**Table 1: Kit Content**

| Component | Intended Use | Amount (10 µL/Rxn) 100 Rxns |
|---|---|---|
| 2X Prime Script Mix | Optimized ready-to-use mix for RT-qPCR assay | 1 x 500 µL |
| HNS Oligo Mix | Specific nucleic acid amplification and detection<br>FAM: Haemophilus influenzae targeting oligonucleotides<br>ROX: Neisseria meningitidis targeting oligonucleotides<br>CY5: Streptococcus pneumoniae targeting oligonucleotides<br>HEX: Internal Control (IC) (RNase P) | 1 x 250 µL |
| NTC | Negative (No Template) Control<br>(Nuclease-free Water) | 1 x 1000 µL |
| PC-HNS | Positive Control (Synthetic RNA fragment mixture of the targets in the "CFR Oligo Mix") | 1 x 250 µL |

**Table 2: Storage Requirements and Shelf Life**

| Component | Transport Conditions | Storage Conditions | Shelf Life |
|---|---|---|---|
| 2X Prime Script Mix | -22 to +8 °C | -22 to -18 °C | 12 months |
| HNS Oligo Mix | | -22 to -18 °C | |
| NTC | | -22 to -18 °C/ +2 to +8 °C | |
| PC-HNS | | -22 to -18 °C before opening, +2 to +8 °C after first thaw | |

**! Each reagent stored at storage temperature, can be used until the expiration date indicated on the tube following the first opening. The expiration date of the kit is determined by the expiration date of the reagents.**

## 2. Materials Required but Not Provided

**Table 3: Components Required but not Included with the Test**

| Components Required but not Included with The Test | |
|---|---|
| **1.** Real-Time PCR instrument with FAM, HEX, ROX and CY5 channels, Ramp rate ≥3°C/sec | **7.** Reaction tubes and their caps/seals compatible with the qPCR instruments and the reaction volume |
| **2.** Adjustable micropipettes and compatible pipette tips (nuclease-free) | **Extra components recommended to use:** |
| **3.** Centrifuge | **8.** Biosafety cabinet for PCR setup |
| **4.** Vortex | **9.** Cold tube rack (for microcentrifuge tubes and PCR tubes/strips) |
| **5.** Nuclease-free water/viral transport medium/serum physiologic | **10.** PPE (Personal Protective Equipment) |
| **6.** 1.5- or 2-mL microcentrifuge tubes (nuclease-free) | |

## 3. Intended Use and Test Principle

The most frequent bacterial pathogens that cause meningitis in adults are *Neisseria meningitidis, Streptococcus pneumoniae,* and *Haemophilus influenzae*. These organisms are spread from person to person by close contact with respiratory secretions.

***Bio-Speedy® H. influenzae/N. meningitidis/S. pneumoniae qPCR Kit*** is designed for the specific identification of *Haemophilus influenzae, Neisseria meningitidis* and *Streptococcus pneumoniae* in cerebrospinal fluid and blood samples from patients with signs and symptoms of meningitis.

The oligonucleotide set targeting the human ***RNase P*** mRNA functions as a control of the sampling, nucleic acid extraction, reverse transcription, and qPCR since the oligonucleotide set targets the exon-exon junction. The kit also contains negative and positive control templates for testing the contamination and the RT-qPCR reagent stability, respectively.

Detection with the kit is achieved via rapid nucleic acid extraction from CSF samples followed by multiplex RT-qPCR targeting the genomic RNA and DNA regions specific to the target agents in real-time PCR instruments that are equipped with FAM, HEX, ROX, and CY5 detection channels. **The kit allows to achieve RT-qPCR result in less than 60 minutes (Run time may vary depending on the instrument)**.

## 4. Analytical Specifications

***Bio-Speedy® H. influenzae/N. meningitidis/S. pneumoniae qPCR Kit*** is validated with robotic extraction system such as ***Zybio EXM3000 Nucleic Acid Isolation System*** *(Model No: EXM3000)* for nucleic acids extracted from CSF samples.

Limit of Detection (LoD) of the kit is between 30-100 copies/mL for CSF samples extracted using the ***Zybio EXM3000 Nucleic Acid Isolation System***.

The qPCR is carried out in 20 µL reaction volume using the ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** Real-Time PCR detection systems equipped with the FAM, HEX, ROX, and CY5 detection channels.

The exclusivity of the kit was tested on 42 different pathogens. The kit does not cross-react with other pathogens. The relative sensitivity and specificity of the kit were determined as 100.00% and 100.00%, respectively.

***Bio-Speedy® H. influenzae/N. meningitidis/S. pneumoniae qPCR Kit*** is intended for use by laboratory personnel trained in the techniques of qPCR and *in vitro* diagnostic procedures.

## 5. Collection, Storage and Shipment of Clinical Specimens

CSF samples should be collected by a healthcare provider in accordance with the specimen collection guidelines. CSF samples are transferred to the laboratory in a sterile transport tube. The samples should be transported to the laboratory within 2 days at 2-8°C. If a delay in shipment is expected, the samples should be frozen at -70°C and shipped with dry ice. It is important that the samples should not be exposed to the repeated freeze-thaw.

## 6. Warnings

1. Specimen processing should be performed in accordance with national biological safety recommendations.
2. Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
3. All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate. Sampling should be carried out by personnel with sufficient knowledge and experience.
4. The kit should be stored away from nucleic acid sources and PCR amplicons.
5. Except for fluid transfers, nucleic acid and positive control tubes should always be kept closed.
6. To prevent contamination of the reaction mixture by previously amplified target sequences, maintain separate work areas, and dedicated equipment.
7. Different sets of laboratory coats should be worn in pre- and post-PCR areas.
8. The micropipettes used for pipetting PCR mixes and template nucleic acids should be separate. Filtered nuclease-free tips should be used.
9. The components in the kit should not be mixed with different lot numbers or chemicals of the same name but from different manufacturers.
10. Master stock reagents should be kept on the cold block during the PCR setup.
11. Kit components should be mixed by gently shaking before use.
12. Maintenance/calibration interval should be determined for all instruments and equipment used with the kit.
13. Immediately after each RT-qPCR run, dispose the qPCR tubes in closed bags to avoid the PCR amplicon contamination in the lab.
14. The wipeable surfaces of the rooms, benches, and devices should be cleaned regularly with freshly diluted 10% bleach solution (0.5% NaClO).
15. Dispose of waste in a designated matter in accordance with local, regional, and federal regulations.

## 7. RT-qPCR Application Protocol

Program the qPCR device as follows and add the reagents into the qPCR tubes, close the tubes, place them into the qPCR instrument and start the run (Table 5).

**Table 5. Reaction Set-up**

| **Reaction Setup** | |
|---|---|
| **Reagent** | **Volume/Rxn** |
| 2X Prime Script Mix | 5 µL |
| Oligo Mix | 2,5 µL |
| Template Nucleic Acid | 2,5 µL |
| TOTAL REACTION VOLUME | 10 µL |

**Table 6. Real-Time PCR Program**

| **RT-qPCR Program** | | | | **QR Code for Thermal Protocol** |
|---|---|---|---|---|
| ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** | | | | |
| **Step** | **Cycle No.** | **Temperature** | **Duration** | |
| **Reverse Transcription** | 1 Cycle | 52 °C | 5 min | |
| **Pre-Incubation** | 1 Cycle | 95 °C | 10 sec | |
| **Denaturation** | 12 Touch Down Cycle: | 95 °C | 1 sec | |
| **Annealing and Extension** | 1 °C decrement in annealing temperature per cycle | 67 °C to 56 °C | 30 sec | |
| **Denaturation** | 35 Cycle | 95 °C | 1 sec | |
| **Annealing and Extension** | | 55 °C | 30 sec | |
| **Detection (Reading)** | | *FAM/HEX/ROX/CY5* | | https://www.bioeksen.com.tr/files/H_influenza_N_meningitidis_S_pneumoniae |

## 8. Interpretation of the Assay Results

- All default analysis options (e.g. **auto-calculated threshold**) in the related software of ***CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS*** instruments should not be changed to calculate Cq values.
- Shape of the amplification curves obtained in the FAM/HEX/ROX/CY5 channels should be examined for all reaction wells returning with Cq values. All the **sigmoidal curves above the threshold** should be recorded as "**positive**" and their Cq values should be recorded. **Non-sigmoidal curves** should be recorded as "**negative**".
- For samples with a sigmoidal curve below the auto-calculated threshold for the ***CFX96 Touch™/CFX96™ Dx (Bio-Rad) and CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments, **the threshold level should be manually set to 200 RFU**. **If the sigmoidal curve exceeds the threshold, the Ct value should be recorded as "35**" and the sample should be reported as **positive**. For samples with a sigmoidal curve below the auto-calculated threshold for ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** should be reported as **positive** and **their Ct values should be recorded as "35"**.
- Because touch down cycles without the fluorescence read were used in the kit, conversion of the detected Ct values to standard cycling values is needed for the reporting. Based on the correlation studies, 12 touch down cycles between 67-56 °C equals to 7 cycles at 55 °C. **Add 7 cycles to the detected Ct value before reporting the Ct values**.

**Table 7. Expected Performance of the Kit Controls**

| Control Type | Control Name | Purpose | Expected Results and Cq Values | |
|---|---|---|---|---|
| | | | ***RNase P* (HEX)** | ***Target* (FAM, HEX, ROX, and CY5)** |
| **Negative Control** | NTC | Contamination control during RT-qPCR | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **No template addition** | NRC | Reagent contamination control | **Not Detected** (No Cq) | **Not Detected** (No Cq) |
| **Positive Control** | PC | Reagent integrity | **Detected** (Cq≤33) | **Detected** (Cq≤33) |
| **Internal/Extraction Control** | IC | To monitor the integrity of nucleic acid extraction and RT-qPCR from each sample | **Detected** (Cq≤33)<br>If IC Cq>33 check the target Cq | If target Cq≤35, conclude it as IC is valid |

If any control does not perform as described above, the run is considered invalid, and the test is repeated.

1. **Invalid PC (Cq>33 in any channel):** It is recommended to contact the manufacturer, renew the reagents, and repeat the reaction.
2. **Invalid NTC (No Cq in any channel):** Repeat the analysis by paying attention to the "**Warnings**" section.
3. **Invalid NRC (No Cq in any channel):** Contact the manufacturer, renew the reagents, and repeat the reaction.
4. **Invalid IC (Cq>33 in HEX channel and no Cq in the other channels):** Repeat the analysis. If the problem continues, then conclude it as an invalid PCR template.

If all the controls are valid, proceed to the interpretation of the results (Table 8).

**Table 8. Test result examples**

| Target | Internal Control | Report | |
|---|---|---|---|
| **Positive (+)** | **Positive (+)** | Report it as **POSITIVE** for the target | 25≤Cq≤35 = Low positive<br>18≤Cq<25 = Positive<br>11≤Cq<18 = High positive<br>Cq<11 = Very high positive |
| **Positive (+)** | **Negative (-)** | Report it as **POSITIVE** for the target | |
| **Negative (-)** | **Positive (+)** | Report it as **NEGATIVE** for the target | |
| **Negative (-)** | **Negative (-)** | **INVALID** Result: Sampling/extraction/inhibition problem<br>Re-extract the specimen and perform testing again. If the result is still invalid, a new specimen should be obtained. If an additional clinical sample is unavailable, report it as **INVALID** | |

**WARNING: On the web page linked with the QR code, examples of the sigmoidal amplification curves are given. The results obtained with this kit should NOT be interpreted without examining these samples.**

## 9. Limitations

- ***Bio-Speedy® H. influenzae/N. meningitidis/S. pneumoniae qPCR Kit*** is intended for use by laboratory personnel trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures.
- The clinical specimens shall be collected by a healthcare provider in accordance with the specimen collection guidelines.
- A false-negative result may occur if a specimen is improperly collected, transported, or handled.
- Mutations within the target regions of the ***Bio-Speedy® H. influenzae/N. meningitidis/S. pneumoniae qPCR Kit*** could affect primer and/or probe binding resulting in failure to detect the presence of virus, bacteria, and fungus.
- Inhibitors or other types of interference may produce a false-negative result. False-negative results may also occur if inadequate numbers of organisms are present in the specimen.

## 10. Explanation of Symbol

| Symbol | Title of Symbol | Symbol | Title of Symbol | Symbol | Title of Symbol |
|---|---|---|---|---|---|
| CE | European Conformity CE Mark | LOT | Batch code | Σ | Contains sufficient for <*n*> tests |
| IVD | *In vitro* diagnostic *medical device* | REF | Catalogue number | | Keep away from sunlight |
| | Manufacturer | NON STERILE | Non-*sterile* | | Protect from heat and radioactive sources |
| | Use-by date | | Consult *instructions for use* or consult electronic *instructions for use* | | Do not use if package is damaged and consult *instructions for use* |
| CONTROL - | Negative control | | Caution | | Keep dry |
| CONTROL + | Positive control | | Temperature limit | | Keep it upright |
| CONTROL | Control | | | | |

## 11. Manufacturer and Technical Support

**Bioeksen R&D Technologies Incorporated Company**
**Address:** Resitpasa Mh. Katar Cd., 4/B-105. 34467, Sariyer, Istanbul, TURKEY.
**Phone:** +90 (212) 285 10 17, **Fax:** +90 (212) 285 10 18
**Web**: www.bioeksen.com.tr, E-**mail:** info@bioeksen.com.tr,
**Technical Support**: support@bioeksen.com.tr

**Notice to User:** Please inform us about product-related incidents at " **vigilance@bioeksen.com.tr** " within 24 hours.

*For in vitro diagnostic use only.*
*For laboratory professional use only.*

**Cat No:** BS-NA-513m-100

# νNAT® Transfer Tube

# Package Insert

## 1. Kit Content

**Table 1: Kit Content**

| Component | Intended Use | Amount |
|---|---|---|
| νNAT® *Transfer Tube* | Microbial nucleic acid storage and stabilization device | 100 tubes |

**Table 2: Storage Requirements and Shelf Life**

| Component | Transport Conditions | Storage Conditions | Shelf Life |
|---|---|---|---|
| νNAT® *Transfer Tube* | +2 to +50 °C | +15 to +30 °C | 18 months |

## 2. Intended Use

νNAT® ***Transfer Tube***, 2 mL of viral nucleic acid extractive and preservative liquid. When clinical specimens suspected of respiratory tract infection are transferred in νNAT® ***Transfer Tube***, the liquid inside the tube can be used directly in Real-Time PCR (qPCR) reactions. The nucleic acid extractive and preservative liquid inactivates all viral, bacterial, or eukaryotic pathogens in the sample, 1 minute after contact with the clinical specimen. The νNAT® ***Transfer Tube*** allows from sample to RT-qPCR in a minute.

## 3. Analytical Specifications

νNAT® ***Transfer Tube*** is validated for RT-qPCR based test kits produced by Bioeksen R&D Technologies Inc.

## 4. Sampling Protocol

Nasopharyngeal swab, oropharyngeal swab, nasal swab, and oral/saliva swab samples shall be collected by a healthcare provider in accordance with the updated version of CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19: https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html. The swab samples should be placed immediately into the νNAT® ***Transfer Tube***. Specimens other than swabs should be extracted with the ***Bio-Speedy®*** νNAT® ***Viral Nucleic Acid Buffer*** (Cat. No: BS-NA-510-100/BS-NA-510-250/BS-NA-510-500/BS-NA-510-1000).

## 5. Sample Transportation, Storage and Analysis Protocol

Store the specimens at +2-8°C and ship to the laboratory on ice pack. If a specimen is frozen at -70°C or lower, ship to the laboratory on dry ice.

Specimens in νNAT® ***Transfer Tube*** can be stored at +2-30 °C for up to 24 hours and +2-8°C for up to 3 months after the collection. If a delay in the RT-qPCR test is expected, store specimens at -70°C or lower in accordance with the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19.

- One minute after the sampling, the samples in the νNAT® ***Transfer Tube*** can directly be used in RT-qPCR.
- Vortex the tube at the highest speed for 3 seconds before adding the sample into the RT-qPCR.

## 6. Explanation of Symbol

| Symbol | Title of Symbol | Symbol | Title of Symbol | Symbol | Title of Symbol |
|---|---|---|---|---|---|
| CE | European Conformity CE Mark | IVD | *In vitro* diagnostic *medical device* | | Keep away from sunlight |
| | Manufacturer | LOT | Batch code | | Protect from heat and radioactive sources |
| | Use-by date | REF | Catalogue number | | Do not use if package is damaged and consult *instructions for use* |
| | Temperature limit | NON STERILE | Non-*sterile* | | Keep dry |
| | Caution | | Consult *instructions for use* or consult electronic *instructions for use* | | |
| | Keep it upright | 2 | Do not re-use | | |

## 7. Manufacturer and Technical Support

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**Web**: www.bioeksen.com.tr, **E-mail:** info@bioeksen.com.tr,
**Technical Support**: support@bioeksen.com.tr

**Notice to User:** Please inform us about product-related incidents at "**vigilance@bioeksen.com.tr**" within 24 hours.