DeepChek® Assay

16S rRNA Bacterial identification

Σ
24

User Guide

Version 2 – Revision 0

For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

REF **131B24** **GTIN: 05407007960798**

Digitally signed by Tudor Bogdan-Mihai
Date: 2024.06.04 10:43:09 EEST
Reason: MoldSign Signature
Location: Moldova

Publication number: IFU_131B24_EN_RUO_V2_R0

May 2023

Document control

Date	Device version	IFU version	Description of change
2023/05/11	B	V2.0	New version of the assay: changes in assay components and in the protocol (amplification regions V1 to V9).
2022/07/20	A	V1.0	Document creation

Contents

Application

For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

The ***DeepChek® Assay 16S rRNA Bacterial identification (RUO)*** is a system which utilizes PCR technology for amplifying relevant portions of the prokaryotic 16S ribosomal RNA (16S rRNA) from input extracted DNA.

This nucleic acid amplification method might aid in the identification or phylogenetic classifications such as genus or species in diverse microbial populations. This test is NOT intended to be used as a screening or confirmation test for the detection, confirmation, and quantification of bacterial infection.

The ***DeepChek® Assay 16S rRNA Bacterial identification (RUO)*** is intended for use by trained laboratory personnel specifically instructed and trained in the techniques of PCR and next generation sequencing (NGS) workflow.

Principles of the assay

The ***DeepChek® Assay 16S rRNA Bacterial identification (RUO)*** is a polymerase chain reaction test which includes primers, reverse and forward, designed to amplify 16S rRNA from input extracted DNA.

16S RNA reverse primers anneal to their respective targets and are extended during a prolonged incubation period. After a denaturation step, in which the temperature of the reaction is raised above the melting point of the double-stranded DNA, a second primer anneals to the DNA strand and is extended by the DNA polymerase activity of the enzyme to create a double-stranded DNA product.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the product is achieved through repeated cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences. The variable V1 to V9 regions of the 16S rRNA are amplified.

The ***DeepChek® Assay 16S rRNA Bacterial identification (RUO)*** is performed on a PCR instrument.

Subsequently, the amplicons can be used for next generation sequencing and analyzed with a downstream analysis software to list in a report 16S RNA identification according to available public reference knowledge databases.

Assay components

The ***DeepChek® Assay 16S rRNA Bacterial identification (RUO)*** is provided in one model of 24 reactions.

Components	Volume for 24 tests	Color cap	Storage
Master Mix	2 x 1120 µL	Green	-25°C to - 15 °C
H_2O	1 x 1200 µL	Blue	-25°C to - 15 °C
R1 (V1+V2) FOR	1 x 40 µL	Yellow	-25°C to - 15 °C
R1 (V1+V2) REV	1 x 40 µL	Red	-25°C to - 15 °C
R2 (V2+V3) FOR	1 x 40 µL	Pink	-25°C to - 15 °C
R2 (V2+V3) REV	1 x 40 µL	Purple	-25°C to - 15 °C
R3 (V3+V4) FOR	1 x 40 µL	Clear	-25°C to - 15 °C
R3 (V3+V4) REV	1 x 40 µL	Brown	-25°C to - 15 °C
R4 (V4+V5) FOR	1 x 40 µL	Orange	-25°C to - 15 °C
R4 (V4+V5) REV	1 x 40 µL	Yellow	-25°C to - 15 °C
R5 (V5+V6) FOR	1 x 40 µL	Green	-25°C to - 15 °C
R5 (V5+V6) REV	1 x 40 µL	Blue	-25°C to - 15 °C
R6 (V7-V9) FOR	1 x 40 µL	Black	-25°C to - 15 °C
R6 (V7-V9) REV	1 x 40 µL	Pink	-25°C to - 15 °C

*Table 1: Components and storage conditions of the DeepChek® Assay 16S rRNA Bacterial identification **V2** (RUO)*

Master Mix	Master Mix	R1 (V1+V2) FOR	R1 (V1+V2) REV	R2 (V2+V3) FOR
R2 (V2+V3) REV	R3 (V3+V4) FOR	R3 (V3+V4) REV	R4 (V4+V5) FOR	R4 (V4+V5) REV
R5 (V5+V6) FOR	R5 (V5+V6) REV	R6 (V7-V9) FOR	R6 (V7-V9) REV	H2O

*Figure 1: Mapping of the assay components for the **131B24 V2** (RUO)*

Reagent storage and handling

The ***DeepChek® Assay 16S rRNA Bacterial identification (RUO)*** is shipped with dry ice and should be maintained and stored immediately upon receipt at –20°C in order to avoid compromising cold chain integrity.
Expiration date: please refer to the label on the kit box.

Materials required but not provided

- Any validated instrument for DNA extraction and purification using magnetic-bead technology.
- PCR instrument e.g. ThermoFisher Scientific Proflex PCR System and associated specific material or any thermal cycler with enough ramp rate of ≥ 1°C/s.
- Benchtop centrifuge with rotor for 0.5 mL/1.5 mL reaction tubes (capable of attaining 10,000 rpm).
- Benchtop vortex mixer.
- Microliter pipets dedicated to PCR (0.1-2.5 µL; 1-10 or 1-20 µL; 20-200 µL; 1000 µL).
- Pipetting Robot (optional).
- Reagents for 0.8–2% agarose gel in 0.5x TBE electrophoresis buffer or equivalent capillary electrophoresis reagent, e.g. Agilent ScreenTape D1000 and Reagents D1000 for Agilent TapeStation 4150.
- Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters.
- Adjustable pipettes & fitting filtered pipette tips.
- Appropriate PPE & workspaces for working with potentially infectious samples.
- Surface decontaminants such as DNAZap (Life Technologies), DNA Away (Thermo Fisher Scientific), RNAse Away (Thermo Fisher Scientific), 10% bleach.
- Nuclease-free dH2O.
- 0.5 ml or 1.5 ml RNase- and DNase-free PCR tubes.
- Ice/Icebox or even cooling blocks.
- 96 well plate cooler (optional).
- 96 well PCR plates.
- Plate thermo seals.
- Plate centrifuge.
- 0.2 mL thin walled 8 tube & domed cap.

Note: Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations. Please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.

Warnings and precautions

- **For Research Use Only (RUO). Not for use in diagnostic procedures.** No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.
- Handle all specimens as of infectious using safe laboratory procedures.
- Store assay reagents as indicated on their individual labels.
- Do not mix reagents from different kit lots.
- Reagents must be stored and handled as specified in these instructions for use. Do not use reagents past the expiration date.
- Work surfaces and pipettes should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNAZap™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.

- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Dispose of waste in compliance with the local, state, and federal regulations.
- Frequent cleaning of the wells of the PCR instrument plate is recommended to prevent contamination.
- To avoid contamination, use separated and segregated working areas.
- Check whether the PCR reaction tubes are tightly closed before loading on the PCR instrument to prevent contamination of the instrument from leaking tubes.

Starting

- Identify the product.
- Verify the expiration date.
- Verify the latest instruction for use available for the product lot number.
- Verify if the product was used already. If yes, check the remaining tests available.

DNA Extraction

To achieve optimal and sensitive DNA analysis, the best representation of the bacterial, it is recommended to extract **fresh samples** for subsequent DNA and amplicon generation and elute in the minimum volume required for your preferred extraction kit.

PCR step-by-step workflow for 16S RNA target

1. Thaw extracted template DNA, primer solutions, Master Mix, RNase-free water and place them on ice. Load all the tubes into the centrifuge. Spin the samples at 11000 g for 10 seconds. Then aspirate and discharge the solution several times before the dispensing.
2. Prepare a master mix according to **Table 2**. The master mix typically contains all the components required for PCR except the template DNA. Prepare a volume of master mix greater (n+1) than required for the total number of reactions to be performed.

Reagent	Volume / Reaction
Master Mix	12.5 µL
R* FOR	1.0 µL
R* REV	1.0 µL
H_2O	5.5 µL
Final volume	**20.0 µL**

**R1 or R2 or R3 or R4 or R5 or R6*

Table 2: Reaction components for the 16S RNA target

3. Vortex the master mix thoroughly and dispense 20µL into PCR tubes. Mix by pipetting the master mix up and down a few times.
4. Add 5µL of DNA to the PCR tubes. Mix by pipetting the master mix up and down a few minutes.

5. Program the thermal cycler according to the program in **Table 3**.

Cycle	Temperature (°C)	Time
Enzyme activation	95	5 min
30 cycles	95	40 sec
	60	2 min
	72	1 min
	10	∞

Table 3: PCR cycling program

6. Start cycling program while PCR tubes are still on ice. After amplification, samples can be stored overnight at 2 – 10°C or at –20°C for long-term storage.
7. [Recommended] - PCR products can be controlled through electrophoresis on an agarose gel. Check the intensity of the signal. Even if low-intensity bands usually lead to a successful sequencing, it is recommended to avoid the process if no band can be observed.

Expected amplicon size:

- **V1+V2: 268bp**
- **V2+V3: 382bp**
- **V3+V4: 372bp**
- **V4+V5: 331bp**
- **V5+V6: 279bp**
- **V7-V9: 379bp**

8. Perform the purification according to the ABL purification protocol and proceed to Library quantification. Use qPCR or Qubit quantification.

9. Perform the library preparation **(116B24 / 116B48 / 116B96 | ABL DeepChek® NGS LIBRARY PREPARATION V2 (24/48/96 reactions** and **124B24 / 124B48 / 124B96|ABL DeepChek® Adapters V2 (24 / 48 / 96).**

Next Generation Sequencing

After the library verification, the samples are ready for the NGS kit processing through Illumina MiSeq.

- **MS-102-303** | MiSeq Reagent Kit v3 (600-cycle)
- **MS-103-1003|** MiSeq Reagent Nano Kit v2 (500-cycles)

NGS data analysis

FastQ NGS files containing nucleotide sequences for metagenomic are analyzed by the **MicrobioChek®** software – Taxonomy 16s module *(ABL, #S-19-MBCKM16).* User shall then follow the **MicrobioChek®** software procedure to complete the data analysis and reporting processes.

Product quality control

In accordance with ABL's Quality Management System, each lot of the assay is tested against predetermined specifications to ensure consistent product quality. Certificates of Analysis are available upon request.

Symbols

 <N>	Contains reagents enough for <N> reactions		Consult instructions for use
	Caution		Temperature limitation
	Catalog number		Serial Number
	Use by	**R*n***	R is for revision of the Instructions for Use (IFU) and n is the revision number
	Manufacturer		Country of manufacturing
	Distributor		

Contact Information

For technical assistance and more information, please see our Technical Support Center at Online: support-diag.ablsa.com; Email: support-diag@ablsa.com; Call +339 7017 0300 Or contact your ABL Field-Application Specialist or your local distributor. For up-to-date licensing information or product-specific disclaimers, see the respective ABL Assay User Guide. ABL User Guides are available at **www.ablsa.com/ifu** or can be requested from ABL Technical Services or your local distributor.

Manufacturer and distributors

Advanced Biological Laboratories (ABL) S.A.
52-54 avenue du X Septembre
2550 Luxembourg, Luxembourg

AdvancedDx Biological Laboratories USA Inc.
5-7 Perry Way, Unit 15 Newburyport, MA 01950, USA

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the device. The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, ABL (S.A) AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Version 2.0

Effective date: 15th May 2023

Digitally signed by Tudor Bogdan-Mihai
Date: 2024.06.04 10:43:36 EEST
Reason: MoldSign Signature
Location: Moldova

EURO-QUALITY SYSTEM France
14 Avenue de l'Europe
77144 MONTEVRAIN

CERTIFICATION SOCIETY

CERTIFICATE

ISO 13485 : 2016

N° Certificate : 221013/1551F

We hereby declare that, on the basis of the dossier of certification, and further to the audit undergone successfully, the company :

Advanced Biological Laboratories (ABL SA) - Luxembourg

Site : ABL Diagnostics S.A

33 Chemin de l'Argile

13010 Marseille, France

has been certified on

7th October 2022

In accordance with the ISO 13485 standard for a period of 3 years in the following fields

- **Design, development, production, distribution, training and support of in-vitro diagnostic medical devices.**
- **Distribution of medical devices.**

and has the right to use this certificate in the fields described before

General Management
Henry CHARLIER

General Management
Christian STIEVENART

Paris,19th October 2022

AN IMPROPER USE OF THIS CERTIFICATE BY THE COMPANY LEADS AUTOMATICALLY TO ITS REVOCATION