ONE LAMBDA
A Thermo Fisher Scientific Brand

One Lambda, Inc.
A Thermo Fisher Scientific Brand
21001 Kittridge Street
Canoga Park, CA 91303
Tel (800) 822-8824
Fax (818) 702-6904
www.onelambda.com

Declaration of Conformity
For
LABType™ DNA Typing Tests

Company:	One Lambda, Inc. 21001 Kittridge Street Canoga Park, CA 91303
Product:	LABType™ DNA Typing Tests - See Declaration of Conformity List (attached)
Classification:	IVDD, Annex II List B (For HLA A, B & DR products) * and IVDD, Non Annex II, Self –Declaration
Conformity assessment Route:	Annex IV of the IVDD (full QA System) * and Annex III (EC Declaration of Conformity)
Certificate Registration No.:	HL 60111158 0001

Digitally signed by Ceaicovschi Tudor
Date: 2021.04.14 16:32:15 EEST
Reason: MoldSign Signature
Location: Moldova

We herewith declare that the above mentioned products are in conformance with the legislation of the Member State for which our Notified Body is located and complies with the In Vitro Diagnostic Medical Devices Directive 98/79/EC.

Notified Body:	TÜV Rheinland LGA Products GmbH Tillystraβe 2, D-90431 Nürnberg Germany
Authorized Representative:	Medical Device Safety Service GmbH Schiffgraben 41 30175 Hannover, Germany

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LABType™ DNA Typing Tests were designed and manufactured in accordance with the following standards: EN ISO 18113-1:2011, EN ISO 18113-2:2011, EN ISO 15223-1:2012, EN ISO 13485:2012, EN 13612:2002, EN ISO 23640:2015, EN 13641:2002, EN 13975:2003, EN 14136:2004, EN ISO 14971:2012, EN ISO 15225:2016, EN 62366-1:2015

SM SM

Sunghee B. Yi
Manager, Regulatory Affairs & Quality Systems

Nov 8, 2017
Date

CALIFORNIA ALL-PURPOSE ACKNOWLEDGMENT **CIVIL CODE § 1189**

A notary public or other officer completing this certificate verifies only the identity of the individual who signed the document to which this certificate is attached, and not the truthfulness, accuracy, or validity of that document.

State of California)
County of LOS ANGELES)

On NOVEMBER 8, 2017 before me, KRISTINE L. KRIEGER
Date *Here Insert Name and Title of the Officer*

Personally appeared SUNGHEE B. YI
Name(s) of Signer(s)

who proved to me on the basis of satisfactory evidence to be the person(s) whose name(s) is/~~are~~ subscribed to the within instrument and acknowledged to me that ~~he~~/she/~~they~~ executed the same in ~~his or~~ her authorized capacity(~~ies~~), and that by ~~his~~/her/~~their~~ signature(s) on the instrument the person(s), or entity upon behalf of which the person(s) acted, executed the instrument.

KRISTINE L. KRIEGER
Commission # 2146281
Notary Public - California
Los Angeles County
My Comm. Expires Mar 14, 2020

Place Notary Seal Above

I certify under PENALTY OF PERJURY under the laws of the State of California that the foregoing paragraph is true and correct.

WITNESS my hand and official seal.

Signature Kristine L. Krieger
Signature of Notary Public

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Declaration of Conformity List

Name of Device, Components, Parts and/or Accessories as per product label	Catalog Number
LABType™ SSO Class II DRB3,4,5 Typing Test LABType™ SSO Class II DRB3,4,5 Typing Test - 20 tests	RSSO2345* RSO2345T*
LABType™ SSO Cass II DRB1 Typing Test LABType™ SSO Class II DRB1 Typing Test - 20 tests	RSSO2B1* RSO2B1T*
LABType™ SSO Class I A Locus Typing Test LABType™ SSO Class I A Locus Typing Test - 20 tests	RSSO1A* RSO1AT*
LABType™ SSO Class B Locus Typing Test LABType™ SSO Class B Locus Typing Test - 20 tests	RSSO1B* RSO1BT*
LABType™ SSO Class I C Locus Typing Test LABType™ SSO Class I C Locus Typing Test - 20 tests	RSSO1C RSO1CT
LABType™ SSO Class I Bw4 Supplement Typing Test LABType™ SSO Class I Bw4 Supplement Typing Test - 20 tests	RSSO1S4* RSO1S4T*
LABType™ SSO MICA	RSSOMICA
LABType™ SSO Class II DQA1/DQB1 Typing Test LABType™ SSO Class II DQA1/DQB1 Typing Test - 20 tests	RSSO2Q RSO2QT
LABType™ SSO Class I B7 Supplement Typing Test LABType™ SSO Class I B7 Supplement Typing Test - 20 test	RSSO1S1* RSO1S1T*
LABType™ HD Class II DRB1 Typing Test LABType™ HD Class II DRB1 Typing Test - 20 tests	RSSOH2B1* RSOH2B1T*
LABType™ HD Class I A Locus Typing Test LABType™ HD Class I A Locus Typing Test – 20 tests	RSSOH1A* RSOH1AT*
LABType™ HD Class I B Locus Typing Test LABType™ HD Class I B Locus Typing Test – 20 tests	RSSOH1B* RSOH1BT*
LABType™ HD Class I C Locus Typing Test LABType™ HD Class I C Locus Typing Test – 20 tests	RSSOH1C RSOH1CT
LABType™ SSO Class I Exon 4-7 Supplement Typing Test LABType™ SSO Class I Exon 4-7 Supplement Typing Test - 20 tests	RSSO1E47 RSO1E47T
LABType™ SSO Class II DPA1/DPB1 Typing Test LABType™ SSO Class II DPA1/DPB1 Typing Test - 20 tests	RSSO2P RSO2PT
Below not included in the kit	
PE - Conjugated Streptavidin	LT-SAPE/ LTSPSAPE (Sample)

Note: The above products are supplied in different quantities. This distinction is designated by "X" or "T" in the catalogue identification number. If the above products are licensed this distinction is designated by "L" in front of the catalogue identification number.

TÜVRheinland®

EC Certificate

Directive 98/79/EC Annex IV, excluding Sections 4 and 6
Full Quality Assurance System
In Vitro Diagnostic Medical Devices

Registration No.: HL 60111158 0001

Report No.: 31593665 001

Manufacturer:
One Lambda, Inc.
21001 Kittridge Street
Canoga Park CA 91303
USA

Products:
In vitro diagnostic assays and reagents for the determination of HLA tissue groups

Products: see attachment

Replaces Approval, Registration No.: HL 60039388 0001

Expiry Date: 2021-06-03

The Notified Body hereby declares that the requirements of Annex IV, excluding section 4 and 6 of the directive 98/79/EC have been met for the listed products. The above named manufacturer has established and applies a quality assurance system, which is subject to periodic surveillance, defined by Annex IV, section 5 of the aforementioned directive. For placing on the market of List A devices covered by this certificate an EC design-examination certificate according to Annex IV, section 4 and a verification of manufactured products according to section 6 is required.

Effective Date: 2016-06-04

Date: 2016-06-02

TÜV Rheinland LGA Products GmbH Zertifizierungsstelle TÜVRheinland®

Notified Body

Dr. H. Lüdemann

TÜV Rheinland LGA Products GmbH - Tillystraße 2 - 90431 Nürnberg

TÜV Rheinland LGA Products GmbH is a Notified Body according to Directive 98/79/EC concerning in vitro diagnostic medical devices with the identification number 0197.

TÜVRheinland®

Doc. 1/1, Rev. 0

TÜV Rheinland
LGA Products GmbH
Tillystraße 2, 90431 Nürnberg

Attachment to Certificate
Registration No.: HL 60111158 0001
Report No.: 31593665 001

Manufacturer:
One Lambda, Inc.
21001 Kittridge Street
Canoga Park CA 91303
USA

Products:

HLA Class I and Class Antigen Typing Products
- AllSet Gold+ SSP
- SeCore Sequence Based Typing
- Micro SSP DNA Typing Tests
- LABType
- HLA Tissue Typing Tests

HLA Antibody Screening
- C1qScreen
- FlowPRA Screening Tests
- LABScreenMulti
- LABScreen
- Lambda Cell Trays
- Lambda Antigen Trays

Monoclonal Antibodies for cell surface antigens
- Fluorescent Conjugated Monclonal Antibodies

TÜV Rheinland LGA Products GmbH TÜVRheinland Zertifizierungsstelle

Notified Body

Date: 2016-06-04

Dr. H. Lüdemann

TÜVRheinland®

TÜV Rheinland
LGA Products GmbH
Tillystraße 2, 90431 Nürnberg

Registration No.: HL 60143129 0001

Report No.: 31593665 001

Manufacturer:
One Lambda, Inc.
22801 Roscoe Blvd
West Hills CA 91304
USA

Products:
HLA Class I and Class Antigen Typing Products
- AllSet Gold+ SSP
- SeCore Sequence Based Typing
- Micro SSP DNA Typing Tests
- LABType
- HLA Tissue Typing Tests
- AllType NGS 11-Loci amplification Kit

HLA Antibody Screening
- C1qScreen
- FlowPRA Screening Tests
- LABScreenMulti
- LABScreen
- Lambda Cell Trays
- Lambda Antigen Trays

Monoclonal Antibodies for cell surface antigens
- Fluorescent Conjugated Monclonal Antibodies

Notified Body

Date: 2019-09-20

TÜVRheinland®

EC Certificate

Directive 98/79/EC Annex IV, excluding Sections 4 and 6
Full Quality Assurance System
In Vitro Diagnostic Medical Devices

Registration No.: HL 60143129 0001

Report No.: 31593665 001

Manufacturer:
One Lambda, Inc.
22801 Roscoe Blvd
West Hills CA 91304
USA

Products:
In vitro diagnostic assays and reagents for the determination of HLA tissue groups

Products: see attachment

Replaces Approval, Registration No.: HL 60111158 0001

Expiry Date: 2021-06-03

The Notified Body hereby declares that the requirements of Annex IV, excluding section 4 and 6 of the directive 98/79/EC have been met for the listed products. The above named manufacturer has established and applies a quality assurance system, which is subject to periodic surveillance, defined by Annex IV, section 5 of the aforementioned directive. For placing on the market of List A devices covered by this certificate an EC design-examination certificate according to Annex IV, section 4 and a verification of manufactured products according to section 6 is required

Notified Body

Effective Date: 2019-09-20

Date: 2019-09-20

TÜV Rheinland LGA Products GmbH - Tillystraße 2 - 90431 Nürnberg

Tel.: +49 221 806-1371 Fax: +49 221 806-3935 e-mail:cert-validity@de.tuv.com http://www.tuv.com/safety

Certificate

The Certification Body of
TÜV Rheinland LGA Products GmbH

hereby certifies that the organization

One Lambda, Inc.
22801 Roscoe Blvd
West Hills CA 91304
USA

has established and applies a quality management system for medical devices for the following scope:

(see attachment for scope and additional site(s) included)

Proof has been furnished that the requirements specified in

EN ISO 13485:2016

are fulfilled. The quality management system is subject to yearly surveillance.

Effective Date:	2019-09-20
Certificate Registration No.:	SX 60143059 0001
An audit was performed. Report No.:	31895531 001
This Certificate is valid until:	2022-06-03

DAkkS
Deutsche
Akkreditierungsstelle
D-ZM-14169-01-02

Certification Body

Date 2019-09-20

TÜV Rheinland LGA Products GmbH - Tillystraße 2 - 90431 Nürnberg

Tel.: +49 221 806-1371 Fax: +49 221 806-3935 e-mail:cert-validity@de.tuv.com http://www.tuv.com/safety

TÜV Rheinland
LGA Products GmbH
Tillystraße 2, 90431 Nürnberg

Attachment to Certificate Registration No.: SX 60143059 0001
Report No.: 31895531 001

Organization:
One Lambda, Inc.
22801 Roscoe Blvd
West Hills CA 91304
USA

Scope:
Design and Development, Manufacturing, Distribution, Servicing and Installation of in-vitro-diagnostic assays, reagents, instruments and software for determination of tissue groups used in the field for compatibility testing for transplantation diagnostics

DAkkS
Deutsche Akkreditierungsstelle
D-ZM-14169-01-02

Certification Body

Date: 2019-09-20

TÜV Rheinland
LGA Products GmbH
Tillystraße 2, 90431 Nürnberg

Attachment to
Certificate
Registration No.: SX 60143059 0001
Report No.: 31895531 001

Organization: One Lambda, Inc.
22801 Roscoe Blvd
West Hills CA 91304
USA

Scope: Additional Site:

One Lambda GmbH
Neuendorfstr. 25
16761 Henningsdorf
Germany

Scope: Activities related to customer service and complaint investigation

Certification Body

DAkkS
Deutsche
Akkreditierungsstelle
D-ZM-14169-01-02

Date: 2019-09-20

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www.onelambda.com

Declaration of Conformity
For
LABScreen™

Company:	One Lambda, Inc. 21001 Kittridge Street Canoga Park, CA 91303
Product:	LABScreen™ - See Declaration of Conformity List (attached)
Classification:	IVDD, Annex II List B (For HLA A, B & DR products) * and IVDD, Non Annex II, Self –Declaration
Conformity assessment Route:	Annex IV of the IVDD (full QA System) * and Annex III (EC Declaration of Conformity)
Certificate Registration No.:	HL 60111158 0001

We herewith declare that the above mentioned products are in conformance with the legislation of the Member State for which our Notified Body is located and complies with the In Vitro Diagnostic Medical Devices Directive 98/79/EC.

Notified Body:	TÜV Rheinland LGA Products GmbH Tillystraβe 2, D-90431 Nürnberg Germany
Authorized Representative:	Medical Device Safety Service GmbH Schiffgraben 41 30175 Hannover, Germany

LABScreen™ was designed and manufactured in accordance with the following standards: EN ISO 18113-1:2011, EN ISO 18113-2:2011, EN ISO 15223-1:2012, EN ISO 13485:2012, EN 13612:2002, EN ISO 23640:2015, EN 13641:2002, EN 13975:2003, EN 14136: 2004, EN ISO 14971:2012, EN ISO 15225:2016, EN 62366-1:2015.

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Declaration of Conformity List

Model or Catalog Number	Product
LS12PRA *	LABScreen™ PRA Class I & II
LS1PRA *	LABScreen™ PRA Class I
LS2PRA *	LABScreen™ PRA Class II
LSM12*	LABScreen™ Mixed Class I & II
LS1A04*	LABScreen™ Single Antigen HLA Class I - Combi
LS2A01*	LABScreen™ Single Antigen HLA Class II - Group 1
LSMICA001	LABScreen™ MICA Single Antigen – Group 1
LS1ASP01*	LABScreen™ Single Antigen HLA Class I Supplement - Group 1
LS2ASP01*	LABScreen™ Single Antigen HLA Class II Supplement - Group 1
Below not included in the kit	
LS-NC	LABScreen Negative Control
LS-AB2	PE-Conjugated Goat Anti-Human IgG

Sunghee B. Yi
Manager, Regulatory Affairs & Quality Systems

Nov 8, 2017
Date

CALIFORNIA ALL-PURPOSE ACKNOWLEDGMENT **CIVIL CODE § 1189**

A notary public or other officer completing this certificate verifies only the identity of the individual who signed the document to which this certificate is attached, and not the truthfulness, accuracy, or validity of that document.

State of California)
County of LOS ANGELES)

On NOVEMBER 8, 2017 before me, KRISTINE L. KRIEGER, NOTARY PUBLIC
Date *Here Insert Name and Title of the Officer*

Personally appeared SUNGHEE B. YI
Name(s) of Signer(s)

who proved to me on the basis of satisfactory evidence to be the person(s) whose name(s) is/~~are~~ subscribed to the within instrument and acknowledged to me that ~~he~~/she/~~they~~ executed the same in ~~his or her~~ authorized capacity(~~ies~~), and that by ~~his~~/her/~~their~~ signature(s) on the instrument the person(s), or entity upon behalf of which the person(s) acted, executed the instrument.

KRISTINE L. KRIEGER
Commission # 2146281
Notary Public - California
Los Angeles County
My Comm. Expires Mar 14, 2020

Place Notary Seal Above

I certify under PENALTY OF PERJURY under the laws of the State of California that the foregoing paragraph is true and correct.

WITNESS my hand and official seal.

Signature Kristine L. Krieger
Signature of Notary Public

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Canoga Park, CA 91303
Tel (800) 822-8824
Fax (818) 702-6904
www.onelambda.com

Declaration of Conformity
For
LABScreen™ Multi

Company:	One Lambda, Inc. 21001 Kittridge Street Canoga Park, CA 91303
Product:	LABScreen™ Multi (LSMUTR, LSMUTRS)
Classification:	IVDD, Annex II List B
Conformity assessment Route:	Annex IV of the IVDD (full QA System)
Certificate Registration No.:	HL 60111158 0001

We herewith declare that the above mentioned products are in conformance with the legislation of the Member State for which our Notified Body is located and complies with the In Vitro Diagnostic Medical Devices Directive 98/79/EC.

Notified Body:	TÜV Rheinland LGA Products GmbH Tillystraße 2, D-90431 Nürnberg Germany
Authorized Representative:	Medical Device Safety Service GmbH Schiffgraben 41 30175 Hannover, Germany

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LABScreen™ Multi was designed and manufactured in accordance with the following standards: EN ISO 15223-1:2012, EN ISO 13485:2012, EN 13612:2002, EN ISO 23640:2015, EN 13641:2002, EN 13975:2003, EN ISO 14971:2012, EN ISO 15225:2016, EN ISO 18113-1:2011, EN ISO 18113-2:2011, EN 62366-1:2015.

Sunghee B. Yi
Manager, Regulatory Affairs & Quality Systems

Nov 8, 2017
Date

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County of LOS ANGELES)

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Personally appeared SUNGHEE B. YI (Name(s) of Signer(s))

who proved to me on the basis of satisfactory evidence to be the person(s) whose name(s) is/~~are~~ subscribed to the within instrument and acknowledged to me that ~~he~~/she/~~they~~ executed the same in ~~his or~~ her authorized capacity(~~ies~~), and that by his/her/~~their~~ signature(s) on the instrument the person(s), or entity upon behalf of which the person(s) acted, executed the instrument.

KRISTINE L. KRIEGER
Commission # 2146281
Notary Public - California
Los Angeles County
My Comm. Expires Mar 14, 2020

Place Notary Seal Above

I certify under PENALITY OF PERJURY under the laws of the State of California that the foregoing paragraph is true and correct.

WITNESS my hand and official seal.

Signature Kristine L. Krieger
Signature of Notary Public

HLA Testing Control Reagents

REF **Catalog # *NS, ABSM, ABSG, AGSM, ATSG, ATSM, ATSMX, ALSG, ALSM***
For Research Use Only. Not for use in diagnostic procedures.

SUMMARY AND EXPLANATION

The Normal Serum Control (OLI Cat. #NS) is used to determine background cell death. The Normal Serum Control is human serum from non-transfused male donors with blood type AB negative.

The Anti-B Cell Controls (OLI Cat. #s ABSM, ABSG) are used to determine the purity of B lymphocytes. The anti-B lymphocyte controls are monoclonal antibodies that are strongly cytotoxic to B lymphocytes with no reactivity against granulocytes, T lymphocytes, platelets, monocytes and red blood cells.

The Anti-Granulocyte Control (OLI Cat. # AGSM) is used to determine the purity of granulocytes. The anti-granulocyte control consists of monoclonal antibodies that are strongly cytotoxic to granulocytes with no reactivity against B lymphocytes, T lymphocytes, platelets, monocytes and red blood cells.

The Anti-Monocyte Control (OLI Cat. # AMSM) is used to determine the purity of monocytes. The anti-monocyte control consists of monoclonal antibodies that are strongly cytotoxic to monocytes with no reactivity against granulocytes, B lymphocytes, T lymphocytes, platelets, and red blood cells.

The Anti-T Lymphocyte Controls (OLI Cat. #s ATSG, ATSM, ATSMX) are used to determine the purity of T lymphocytes. The anti-T lymphocyte controls are monoclonal antibodies that are strongly cytotoxic to T lymphocytes with no reactivity against granulocytes, monocytes, B lymphocytes, platelets and red blood cells.
Note: *ATSMX is the only anti-T control for use with FluoroBeads® T-isolated cells.*

The Anti-Lymphocyte Controls (OLI Cat. #s ALSG, ALSM) are used to determine complement reactivity. The anti-Lymphocyte controls are monoclonal antibodies that are strongly cytotoxic to human lymphocytes with no reactivity against granulocytes, monocytes, platelets, and red blood cells.

Note: DTT is known to deactivate IgM, but not IgG. *Do not use ALSM, ALSG, ATSG, ATSM, or ATSMX as a control for testing with Dithiothreitol (DTT), 5Mm DTT used to deactivate human IgM does not deactivate ALSM, ATSM, and ATSMX because they are mouse monoclonal IgM antibodies and their disulfide bonds are not broken by DTT.*

Viable lymphocytes are incubated with complement-binding antibody. If the lymphocytes express an antigen recognized by a specific antibody, the Fab portion of the antibody binds to the antigen forming antigen-antibody complex. After these complexes have formed, rabbit complement is added. The C1q and Ca^{++} from the complement bind to the FC portion of the antibody. One IgM antibody is required to bind one molecule of C1q or two IgG antibodies are required to bind one molecule of C1q. Binding of C1q with antigen-antibody complexes initiates the complement cascade that leads to cell lysis. In a negative reaction, the lymphocytes are alive. In a positive reaction, the lymphocytes are dead.

REAGENTS

A. Identification

Testing Control Reagents are supplied frozen (1ml). Specificity and dilution factors are determined by the microcytotoxicity test using NIH standard conditions. Subtypes: Catalog numbers with an “M” indicate the antibody is IgM, and those with a “G” indicate the antibody is IgG.

B. Warning or Caution
   1. **Warning/Caution**: All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.
   2. Refer to the Material Safety Data Sheet for detailed information.

C. Preparing Reagents for Use
   1. See “Directions for Use.”

D. Storage Instructions

   Store reagents at temperature indicated on package. Use before printed expiration date. Avoid high temperature. ***Avoid repeated freezing and thawing of control reagents.***

E. Purification or Treatment Required for Use

   See “Directions for Use.”

F. Instability Indications

   Do not use if antibody is not stored properly.

SPECIMEN COLLECTION AND PREPARATION

A. Since viable lymphocytes are required for serological typing, blood should be received and processed immediately following procurement. Lymphocyte yield decreases with time and extreme temperature. Blood should be collected in acid citrate dextrose (ACD) or sodium heparin, stored horizontally at room temperature (20 - 25°C), and processed within 48 hours for maximum T and B lymphocyte yield.

PROCEDURE

A. Materials Provided
   1. Vial of HLA Testing Control Reagent
   2. Instructions for Use

B. Recommendations for Use
   1. Frozen Reagents
      a. Thaw in cold tap water.
      b. Frozen control reagents are provided at cytotoxic working dilutions for immediate use.

RESULTS

None. Product is for Research Use Only.

LIMITATIONS OF THE PROCEDURE

- Erroneous results may occur when cell concentrations are above or below acceptable levels. Bacterial contamination or change in pH of the antisera may cause false negative reactions.
- Product is for research use only and not to be used in diagnostic procedures.

EXPECTED VALUES

None. Product is for Research Use Only.

SPECIFIC PERFORMANCE CHARACTERISTICS

None. Product is for Research Use Only.

EXPLANATION OF SYMBOLS

Symbol	Description
REF	Catalogue number
	Consult accompanying documents
	Temperature limitation
	Manufacturer

REVISION HISTORY

Revision	Date	Revision Description
11	2014/08	Update to new template. Updated Summary and Explanation section.
01	04/08/2019	Upgraded Internal Document Control System. No changes to the document content.
02	Current	Updated contact information and address to reflect change in legal manufacture site.

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21001 Kittridge Street, Canoga Park, CA 91303-2801 Tel: (818) 702-0042 Fax: (818) 702-6904 WEB:www.onelambda.com

PRODUCT INSERT

REF

CLASS I AND CLASS II COMPLEMENT

Catalog #s CABC-5, CABC-50, CDR5, CDR50, CABC-1D, and CDR-1D

Important: *Instructions in this product insert must be used in conjunction with the product insert for Terasaki HLA Tissue Typing Trays. Complement is used in the preparation of cytotoxicity assays.*

IVD **For In Vitro Diagnostic Use.**

INTENDED USE

For use in complement dependent cytotoxicity assays for determining HLA Class I and Class II cell surface antigens.

SUMMARY AND EXPLANATION

Rabbit serum is a source for complement for the microcytoxicity test. ABC complement is used for HLA Class I typing and DR complement is used for Class II typing. Each complement has been titrated against a panel of Class I or Class II typing reagents for potency and non-cytotoxicity against T and B lymphocytes.

PRINCIPLE(S)

Viable lymphocytes are incubated with complement-binding antibody. If the lymphocytes express an antigen recognized by a specific antibody, the Fab portion of the antibody binds to the antigen forming antigen-antibody complex. After these complexes have formed, rabbit complement is added. The C1q and Ca ++ from the complement binds to the FC portion of the antibody. One IgM antibody is required to bind one molecule of C1q or two IgG antibodies are required to bind one molecule of C1q. Binding of C1q with antigen-antibody complexes initiates the complement cascade that leads to cell lysis. In a negative reaction, the lymphocytes are alive. In a positive reaction, the lymphocytes are dead.

REAGENTS

A. Identification
Rabbit complement is frozen or lyophilized. Frozen complement is packaged in 5 ml and 50 ml volumes. Lyophilized complement volume is
1 ml reconstituted.

B. Warning or Caution
1. For In Vitro Diagnostic Use.
2. Refer to the Material Safety Data Sheet for detailed information.

C. Instructions for Use
See “Directions for Use.”

D. Storage Instructions
Frozen complement should be stored at a temperature of -65º degrees or colder. Lyophilized complement can be stored at 2 - 5º C until used. Use before expiration date printed on package.

E. Purification or Treatment Required for Use
See “Directions for Use.”

F. Instability Indications
Complement stability is affected by heat. Therefore, if frozen complement is received thawed, discard complement.

SPECIMEN COLLECTION AND PREPARATION

See product insert for Terasaki HLA Tissue Typing Trays.

PROCEDURE

A. Materials Provided
HLA Class I or Class II complement, frozen or lyophilized.

B. Materials Required, But Not Provided
Terasaki Tissue Typing Trays

C. Step-by-step procedure.
See “Directions For Use” below.

DIRECTIONS FOR USE

A. Frozen Complement
1. Before use, frozen complement should be thawed in a water bath at a temperature of 20º C or in cool tap water. Remove thawed complement immediately and place in a container filled with crushed ice.
2. Do not freeze/thaw more than once after initial thaw.

B. Lyophilized Complement Reconstitution
1. Add 1 ml sterile water at 2 -5º C to each vial of lyophilized complement.
2. Mix gently until full dissolved.
3. Store at 2 –5º C until use.
4. Unused complement must be aliquoted and immediately stored at -20º C or below. Do not freeze-thaw more than once after reconstitution.

RESULTS

Refer to product insert for Terasaki HLA Tissue Typing Trays.

LIMITATIONS OF THE PROCEDURE

Refer to product insert for Terasaki HLA Tissue Typing Trays.

EXPECTED VALUES

Refer to product insert for Terasaki HLA Tissue Typing Trays.

SPECIFIC PERFORMANCE CHARACTERISTICS

Refer to product insert for Terasaki HLA Tissue Typing Trays.

BIBLIOGRAPHY

Refer to product insert for Terasaki HLA Tissue Typing Trays.

EC REP EUROPEAN AUTHORIZED REPRESENTATIVE

MDSS GMbH, Burckhardstrasse 1, D-30163, Hannover, Germany

CE 0197

REVISION HISTORY

Revision	Date	Revision Description
1	2005/04	Add symbols for translation.

LABScreen™ Negative Control

REF **Catalog # LS-NC**

IVD In Vitro Diagnostic Medical Device.

INTENDED USE

This reagent is for use as an indicator of the non-specific background signal of each LABScreen™ and LABScreen™ Multi* bead (HLA-coated beads, as well as negative and positive control beads) when reacting with a serum sample that does not contain any anti-HLA Class I or Class II antibody. This reagent may only be used with LABScreen™ products.

SUMMARY AND EXPLANATION

The different antigen-coated bead preparations included in the LABScreen™ and LABScreen™ Multi* products may exhibit variable degrees of non-specific binding by test sera or by the secondary antibody indicator. This may also be influenced by technical variables, such as the stringency of the bead washing procedure, or the calibration of the flow analyzer. To correct for the resulting differences in fluorescent signal of the assay, all test data is normalized to a negative control serum run on the same day and corrected for binding to a non-HLA-coated negative control bead, as well.

PRINCIPLE(S)

The Negative Control Serum is incubated with the LABScreen™ and LABScreen™ Multi* beads in parallel with a batch of test sera. The median fluorescent value of each test serum for each bead is adjusted by dividing by the corresponding median fluorescent value of the negative control serum versus that same bead. This allows for calculation of a ratio to express the relative strength of a given sample in the binding assay relative to a known negative. It also corrects for non-antibody serum factors that may result in different levels of background fluorescence on each bead.

REAGENTS

A. Identification

- Amount Provided: 400 µl per vial (20 tests)
- Physical State: Frozen
- Source: A pool of undiluted negative serum samples drawn from 5 to 10 non-transplanted and non-transfused male blood donors
- Pretreatment: Defibrination, high speed centrifugation and 0.25µm filtration.
- Preservatives: None

IVD B. Warning or Caution

1. In Vitro Diagnostic Medical Device.
2. Warning: All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.
3. **Warning:** The LABScreen™ Negative Control Serum contains 0.1% sodium azide (NaN_3) as a preservative. Under acidic conditions, sodium azide yields hydrazonic acid, an extremely toxic compound. Reagents containing sodium azide should be diluted in running water prior to being discarded. These conditions are recommended to avoid deposits in plumbing where explosive conditions may develop.
4. Refer to the Material Safety Data Sheet for detailed information.

One Lambda, Inc.
22801 Roscoe Blvd. West Hills, CA 91304 U.S.A
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C. Instructions for Use
   1. Mix by inversion and tapping of vial after thawing and before each use.

D. Storage Instructions
   1. Store at -20° C or below until first usage. After thawing keep at 2 - 8°C for 5 days. Freeze aliquots, or re-freeze the original reagent vial (1X only) if reagent is not used within 5 days.
   2. Use before printed expiration date.

E. Purification or Treatment Required for Use

   None

F. Instability Indications

   Cloudiness or precipitate may indicate microbial contamination has been introduced. Do not use if this occurs.

INSTRUMENT REQUIREMENTS

Not applicable.

SPECIMEN COLLECTION AND PREPARATION

Not applicable.

PROCEDURE

A. Materials Provided
1. Serum containing no anti-HLA antibody when tested by LABScreen™ assay

B. Materials Required, But Not Provided
1. LABScreen™ products for HLA antibody detection

C. Step-by-step procedure
1. See DIRECTIONS FOR USE below

D. Directions for Use
1. See “Directions For Use” in the LABScreen™ product insert.
2. Use 20 µl of negative control serum per test.
3. Incubate with selected LABScreen™ test beads according to the assay protocol.

RESULTS

A. Data Acquisition
1. See the LABScreen™ product insert.

B. Data Analysis
1. See the LABScreen™ product insert.

LIMITATIONS OF THE PROCEDURE

See the LABScreen™ product insert.

*CE mark does not apply to LABScreen Multi product.

EXPECTED VALUES

The expected fluorescent values for the binding of LS-NC to each of the LABScreen™ assay beads is provided in lot-specific data sheets for each of the LABScreen products.

SPECIFIC PERFORMANCE CHARACTERISTICS

The negative control serum affords a standardized benchmark for the performance of the LABScreen™ assay. It is used to establish a ratio of signal (test serum) to background (LS-NC) for each bead in a test batch.

BIBLIOGRAPHY

Not applicable.

TRADEMARKS AND DISCLAIMERS

™LABScreen is a trademark of One Lambda, Inc.

EUROPEAN AUTHORIZED REPRESENTATIVE

EC REP MDSS GmbH, Schiffgraben 41, 30175, Hannover, Germany

EXPLANATION OF SYMBOLS *(reference EN ISO 15223-1: Medical devices – Symbols to be used with medical device labels, labeling and information to be supplied)*

Symbol	Description
REF *ISO 7000 Reg No. 2493*	Catalog number
IVD	In vitro diagnostic medical device
 *ISO 7000 Reg No. 1641*	Consult instructions for use
 *ISO 7000 Reg No. 0434A*	Caution, consult accompanying documents
 *ISO 7000 Reg No. 0659*	Biological risks
 *ISO 7000 Reg No. 0632*	Temperature limitation
 *ISO 7000 Reg No. 0633*	Upper limit of Temperature
 *ISO 7000 Reg No. 3082*	Manufacturer
EC REP	Authorized representative in the European Community
 *ISO 7000 Reg No 2497*	Date of Manufacture
LOT	Batch Code
 *ISO 7000 Reg No 2607*	Use By Date

Batch field on the label is for traceability of manufacturing event

REVISION HISTORY

Revision	Date	Revision Description
02	21 Sep 2019	Updated contact information and address to reflect change in legal manufacture site.
03	01 Feb 2021	Update Explanation of Symbols table.
04	Current	Update IVD verbiage on page 1 to align with symbols table.

CE

Certificate of Quality

Luminex® 100/200 Calibration Kit LX200-CAL-K25

REF	Product Number	LX200-CAL-K25
LOT	Lot Number	
	Kit Expiration Date	2021-05-19

Kit Components	**Part Number**	**Lot***
xMAP Classification Calibrator, Microspheres, 5 mL	L100-CAL1	B75346
MagPlex Classification Calibrator, Microspheres, 5 mL	MCAL1-05	B74612
xMAP Reporter Calibrator, Microspheres, 5 mL	L100-CAL2	B75279
8-Well Microtiter plate strips, Pack of 25	13-52047	N/A
LX100/200 Calibrator Kit CD (contains Kit Lot Importable File, component CoQs, MSDS and Kit Instructions)	89-20191-00-001	

This certificate provides descriptive lot information and assurance that quality requirements have been met for the components, intermediates, and finished product. Luminex Corporation certifies that the Luminex 100/200 Calibration Kit, LX200-CAL-K25, lot defined above conforms to all prescribed acceptance criteria and is fit for its intended use.

*Reagent target values may vary lot to lot. Only the reagent lots listed should be used when performing system calibration with this kit lot.

Luminex®

EN ISO 13485 **C**ertified Company

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Certificate of Quality

Luminex® 100/200 Performance Verification Kit LX200-CON-K25

REF	Product Number	LX200-CON-K25
LOT	Lot Number	
	Kit Expiration Date	2022-03-10

Kit Components	Part Number	Lot*
xMAP Classification Control, Microspheres, 5 mL	L100-CON1	B80929
MagPlex Classification Control, Microspheres, 5 mL	MCON1-05	B79784
xMAP Reporter Control, Microspheres, 5 mL	L100-CON2	B80031
xMAP Fluidics 1, Microspheres, 5 mL	FLUID1-05	B80930
xMAP Fluidics 2, Microspheres, 5 mL	FLUID2-05	B80931
8-Well Microtiter plate strips, Pack of 25	13-52047	N/A
LX100/200 Performance Verification Kit CD (contains Kit Lot Importable File, component CoQs, MSDS and Kit Instructions)	89-20192-00-001	

This certificate provides descriptive lot information and assurance that quality requirements have been met for the components, intermediates, and finished product. Luminex Corporation certifies that the Luminex 100/200 Performance Verification Kit, LX200-CON-K25, lot defined above conforms to all prescribed acceptance criteria and is fit for its intended use.

*Reagent target values may vary lot to lot. Only the reagent lots listed should be used when performing system verification with this kit lot.

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LABScreen™

REF

Catalog ID	Product Name
LS1PRA*	LABScreen™ PRA Class I
LS2PRA*	LABScreen™ PRA Class II
LS12PRA*	LABScreen™ PRA Class I & II
LSM12*	LABScreen™ Mixed Class I & II
LS1A04*	LABScreen™ Single Antigen HLA Class I - Combi
LS1ASP01*	LABScreen™ Single Antigen HLA Class I Supplement - Group 1
LS2A01*	LABScreen™ Single Antigen HLA Class II - Group 1
LS2ASP01*	LABScreen™ Single Antigen HLA Class II Supplement - Group 1
LS1AEX01*	LABScreen™ Single Antigen HLA Class I ExPlex
LS2AEX01*	LABScreen™ Single Antigen HLA Class II ExPlex
LSMICA001	LABScreen™ MICA Single Antigen - Group 1
LSPWABUF	LABScreen™ Wash Buffer

IVD For In Vitro Diagnostic Use.

INTENDED USE

LABScreen products are intended for use in detection of HLA antibody using flow cytometric technology

SUMMARY AND EXPLANATION

LABScreen products use microbeads coated with purified Class I or Class II HLA antigens and pre-optimized reagents for the detection of Class I or Class II HLA antibodies in human sera. LABScreen products utilize the LABScan™ 100 (Luminex® 100/200) or LABScan3D™ (Luminex® FLEXMAP 3D®) for analysis of up to 100 or 500 bead regions, respectively, in a single test.

The Mixed assay detects the presence of antibody to Class I and/or Class II HLA antigens. The PRA tests can detect antibodies and their specificities against the HLA antigens in each LABScreen panel. The Single Antigen assay allows confirmation of antibody specificity suggested by a previous PRA test, while individual Singles beads are used to focus on reactions against one or a few antigens, e.g. to compare reactivity of different serum samples from the same individual. A negative control serum is used to establish the background value for each bead in a test batch.

PRINCIPLE(S)

Test serum is incubated with LABScreen beads. Any HLA antibodies present in the test serum bind to the antigens on the beads and then are labeled with R-Phycoerythrin (PE)-conjugated goat anti-human IgG. The LABScan™ 100 or LABScan3D™ flow analyzer(s) simultaneously detects the fluorescent emission of PE and a dye signature from each bead, allowing almost real-time data acquisition. To assign PRA and HLA specificity, the reaction pattern of the test serum is compared to the lot-specific worksheet defining the antigen array.

REAGENTS

A. Identification

See LABScreen Reference Table for product description.

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B. Warning or Caution

1. **Warning**: LABScreen PRA test reagents contain 0.1% sodium azide (NaN_3) as a preservative. Under acidic conditions, sodium azide yields hydrazoic acid, an extremely toxic compound. Dilute reagents containing sodium azide in running water before discarding, to avoid deposits in plumbing where explosive conditions may develop. (Refer to Material Safety Data Sheet for detail.)
2. **Warning**: All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.
3. **Caution**: For manual flicking of trays, use a quick downward arm motion without wrist movement to prevent repetitive motion effects.
4. Refer to the Material Safety Data Sheet for detailed information.

C. Preparing Reagents for Use

1. See Directions for Use, below.
2. If buffer salts have precipitated out of solution during shipment or storage, re-dissolve by gently warming before preparing working dilution.

D. Storage Instructions

1. LABScreen products are shipped to the end user on dry ice. The entire package may be stored in a freezer at -65° C or below until first use, up to the labeled expiration date.
2. Once beads are thawed, DO NOT REFREEZE. Store at 2 - 8° C for up to three months or until the expiration date (if earlier).
3. After first use, store wash buffer at 2 - 8°C for up to three months or until the expiration date, if earlier.

E. Purification or Treatment Required for Use

See Directions for Use, below.

F. Instability Indications

None

INSTRUMENT REQUIREMENTS

A. Required Equipment

- LABScan 100 flow analyzer (Luminex® 100/200) with Luminex® XY platform (for automated 96-sample data acquisition) and sheath fluid delivery system (OLI Cat. # LABSCNXS3) OR LABScan3D flow analyzer (Luminex® FLEXMAP 3D®) with XY platform and sheath fluid delivery system (OLI Cat. # LABSCNXS4)
- Centrifuge
- Rotor for 1.5 ml microcentrifuge tube (9,300 g), or a swinging bucket rotor for 96-well microplate (1,300 g)
- Vortex mixer
- Plate shaker or rotating platform

For Filter Plate Option:

- Vacuum manifold, 96-well (Millipore Cat. # MAVM0960R or equivalent)
- Vacuum pump with a pressure less than 100 mm Hg
- Plate shaker or rotating platform

B. Equipment Calibration

Follow manufacturer's instructions for calibration of the LABScan 100 or LABScan3D flow analyzer.

C. Recommended Software

HLA Fusion™ (OLI Cat. # FUSPGR)

SPECIMEN COLLECTION AND PREPARATION

- Unopened blood specimens may be kept at room temperature up to 4 days. Separated serum (from clotted samples) or plasma (in ACD or K-EDTA) may be refrigerated up to 7 days, or aliquots may be frozen at -20°C or below and thawed just before the assay. Aggregates should be removed from the test serum/plasma by centrifugation (8,000 – 10,000 g for 10 minutes) or filtration (0.2 µm) prior to testing. Any aggregates or contamination of the sample may generate invalid results.
- Samples may be treated or diluted to reduce non-specific background or to remove inhibitory factor – see limitation section.

  Note:

  - Test serum or plasma should not be heat inactivated, because it may give a high background in the test.
  - Undiluted serum or plasma is generally used for the test. However, if a high background serum sample is diluted for this assay, the negative control serum should be tested at the same dilution.

PROCEDURE

A. Materials Provided

1. See the LABScreen Reference Table in Product Documentation on the One Lambda, Inc. web site for a list of materials provided for each product.
2. The volumes provided exceed the amount required for testing by a small amount to allow for pipetting losses.

B. Materials Required, But Not Provided

1. PE–Conjugated Goat Anti-Human IgG (OLI Cat. # LS-AB2)
2. PBS, filtered [USA Scientific Cat. # 9242 (500 ml 10X) or equivalent]
3. 1.5 ml microcentrifuge tube (USA Scientific Cat. # 1415-2500 or equivalent)
4. Pipette tips (Rainin GPS)
5. Negative Control Serum, containing no HLA antibody when tested by LABScreen method (OLI Cat. # LS-NC or equivalent)

If the test is performed in a 96-well microplate:

1. 96-well microplate, 250 µl, non-treated surface (Whatman Cat. # 7701-3250 or equivalent)
2. Tray seal (OLI Cat. # SSPSEA300 or equivalent)

For the Filter Plate option:

1. Filter plate (Multiscreen-BV, Millipore Cat. No. MABVN1250 or equivalent)

C. Directions for Use

Notes:

- Take special care in the aliquoting process. Failure to follow the steps described below may result in reagent loss.
- Sections A through C indicate the volumes of reagents needed for testing a single bead group. If you are running a combined test, see Section D before proceeding.
- Turn on the LABScan 100 or LABScan3D flow analyzer at least 30 minutes before starting the assay.
- Create a filename and sample code sheet for each test tray.

I. For each test batch, test a negative control serum (e.g. OLI Cat. # LS-NC or equivalent) to establish background values. To complete the test in a 1.5 ml microcentrifuge tube

1. Mix the LABScreen beads well by gently vortexing or pipetting up and down several times prior to use.
2. Incubate 5 µl of LABScreen beads with 20 µl of test serum in a 1.5 ml micro-centrifuge tube for 30 minutes, in the dark at 20 - 25º C with gentle shaking.
3. Dilute 10X wash buffer (OLI Cat. # LSPWABUF) in distilled water to make a 1X solution.
4. Add 1 ml of 1X wash buffer to each bead/serum solution tube and vortex. Centrifuge at 9,300 g for 2 minutes. Aspirate and discard the supernatant.
5. Repeat Step 4 twice.
6. Dilute 1 µl per test of 100X PE-conjugated anti-human IgG (OLI Cat. # LS-AB2) with 99 µl of 1X wash buffer to make a 1X solution.
7. Add 100 µl of 1X PE-conjugated anti-human IgG to each tube. Vortex and then incubate in the dark for 30 minutes at 20 - 25º C with gentle shaking.
8. Repeat Step 4 twice.
9. Add 80 µl 1X PBS to each tube. Proceed to data acquisition and analysis, or store tray at 2 - 8º C in the dark for up to 24 hours before analysis.

II. To complete the test in a 96-well plate

Caution: Seal the 96-well tray carefully and completely to prevent well-to-well sample contamination by pressing the seal against each rim of the 96 wells. Do not re-use tray seals. Use a fresh seal for each step that requires application of a tray seal.

1. Mix the LABScreen beads well by gently vortexing or pipetting up and down several times prior to use.
2. Add 5 µl of LABScreen beads with 20 µl of test serum in each well of a 96-well plate, seal plate, and incubate for 30 minutes in the dark at 20 - 25º C with gentle shaking.
3. Dilute 10X wash buffer (Cat. # LSPWABUF) in distilled water to make a 1X wash solution.
4. After incubation, remove seal and add 150 µl of 1X wash buffer to each well of the plate. Cover with tray seal (OLI Cat. # SSPSEA300 or equivalent) and vortex. Centrifuge at 1,300 g for 5 minutes.
5. Remove wash buffer from wells of plate by flicking or with vacuum aspiration.
6. Add 200 µl of 1X wash buffer to each well of the plate. Cover with a new tray seal and vortex. Centrifuge at 1,300 g for 5 minutes.
7. Remove supernatant from wells of plate by flicking or with vacuum aspiration.
8. Repeat Steps 6 and 7.
9. Dilute 1 µl per test of 100X PE-conjugated anti-human IgG (OLI Cat. # LS-AB2) with 99 µl of 1X wash buffer to make a 1X solution.
10. Add 100 µl of 1X PE-conjugated anti-human IgG to each well. Cover with tray seal and vortex. Incubate in the dark for 30 minutes at 20 - 25º C with gentle shaking.
11. Centrifuge at 1,300 g for 5 minutes.
12. Remove supernatant from wells of plate by flicking or with vacuum aspiration.
13. Repeat Steps 6 and 7 twice.
14. Add 80 µl 1X PBS to each well. Cover with a new tray seal and vortex. Proceed to data acquisition and analysis, or store tray at 2 - 8º C in the dark for up to 24 hours before analysis.

III. To complete the test in a 96-well filter plate

1. Mix the LABScreen beads well by gently vortexing or pipetting up and down several times prior to use.

2. Dilute 10X wash buffer (OLI Cat. # LSPWABUF) in distilled water to make a 1X solution (approximately 3.2 ml/tray/wash).
3. Cover any wells of the plate that will remain unused during the test with a tray seal to assure that the unused wells remain dry. Pre-wet filters in the filter plate by dispensing 300 µl wash buffer into only those wells that will be used for the assay.
4. Incubate the plate for 10 minutes on a platform plate shaker at low speed.
5. Aspirate all wash buffers from the wells using a Millipore vacuum manifold. Do not exceed 100 mm Hg vacuum pressure!
6. Add 5 µl of LABScreen beads and 20 µl of test serum per test well.

   Note: During bead and sample dispensing steps, press pipette tip gently against filter plate well to avoid filter rupture.
7. Incubate the plate in the dark for 30 minutes at 20 - 25º C with gentle shaking.
8. Add 175 µl wash buffer per well.
9. Turn on vacuum pump. Press the plate firmly on the vacuum manifold. Make sure liquid drains out slowly. Make sure all liquid has drained from the wells before proceeding.

⚠ **Caution**: Do not exceed 100 mm Hg vacuum pressure. A rapid vacuum will cause loss of beads due to be entrapment in the pores of the filter paper.

10. Repeat Steps 8 and 9, above, four times.
11. Add 100 µl of 1X PE-conjugated anti-human IgG to each well.
12. Incubate in the dark for 30 minutes at 20 - 25º C with gentle shaking.
13. Repeat Steps 8 and 9 five times.
14. Add 80 µl of 1X PBS to each well.
15. Read sample on the LABScan 100 or LABScan3D flow analyzer, adjusting probe height if necessary.

    Note: LABScreen Single Antigen HLA Class I and Class II ExPlex products can be used on the LABScan3D flow analyzer only.

IV. Combined tests

Any of the above protocols can be used for a combined test of certain LABScreen products.

- For acceptable lot combinations of LS12PRA see www.onelambda.com
  (Antibody Detection>LABScreen>LABScreen PRA/ Product Documentation: LABScreen Bead Combo – Multiple IDs DataSheet).
- Do not combine LABScreen Single Antigen Class I Combi and Class II panels (bead IDs would overlap).

1. Mix equal volumes of beads. Then dispense the appropriate aggregate amount (10 or 15 µl) of bead mixture per test.
2. Bead combinations and amounts to dispense are listed in the table below.

Catalog ID	Bead volume per Test	Control (NC/PC) Beads	Test Serum per Test	Instrument
LS12PRA (CI and CII beads)	5 µl + 5 µl	Included	40 µl	LABScan 100 or LABScan3D
LS1A04 + LS1AEX01	5 µl + 5 µl	Included	40 µl	LABScan3D
LS2A01 + LS2AEX01	5 µl + 5 µl	Included	40 µl	LABScan3D
LS1A04	5 µl	Included	20 µl	LABScan 100 or LABScan3D

RESULTS

A. Data Acquisition

1. Set up the LABScan 100 or LABScan3D flow analyzer for sample acquisition and calibration according to the Luminex User's Manual.[1]
2. Choose a template according to product kit catalog ID and lot number.
   a. Acquisition templates are available from OLI by CD or via our download website.
   b. To create your own acquisition template, follow instructions in the Acquisition chapter of the Luminex User's Manual.
   c. Luminex software versions - LABScan 100 (xPONENT 3.1 or higher); LABScan 3D (xPONENT 4.2 or higher) must be used.
3. Create a file name for the samples to be run.
4. Make sure all the template settings are correct. Template specifications are:
   a. Set sample volume to 50 µl.
   b. Set sample time-out to 80 seconds.
   c. Set doublet discriminator gate to 8,000 (low limit) and 16,000 (high limit).
   d. Set number and ID of beads selected according to the product-specific worksheet provided with the product.
   e. Set minimum events collected to 100 per bead.
5. Enter the sample IDs (if the same sample is tested more than once, assign a different ID).
6. Load the plate onto the XY platform and fill the reservoir with sheath fluid.
7. Click the START button to initiate the session. After the samples have finished running, save the data output in a .csv file.
8. Wash the machine twice with sheath fluid at the end of the session.

B. Data Analysis

1. The reactivity of a test sample is calculated from the "raw" fluorescence values recorded by the LABScan device (.csv file) for each HLA coated bead.
2. Calculate anti-HLA serum reactivity by correcting for non-specific binding to the negative control bead and background values (obtained by testing with a negative control serum (OLI Cat. # LS-NC) to determine the normalized background ratio (NBG ratio). See Calculations, below.
3. For LABScreen PRA or LABScreen Single Antigen, the normalized fluorescent value for each HLA coated bead equals the value of that bead divided by the value of the NC bead. (For LABScreen Mixed, the normalized fluorescent signal equals the value of the Class I or Class II coated bead minus the value of the NC bead.)

   Note: The fluorescent signal (value) can be either the trimmed mean or median value.

C. Calculations

1. The abbreviations used in this section are defined below:

NBG ratio	Normalized Background ratio used to assign strength of each anti-HLA reaction
S#N	Sample-specific fluorescent value for bead #N
SNC bead	Sample-specific fluorescent value for Negative Control bead
BG#N	Background NC Serum fluorescent value for bead #N
BGNC bead	Background NC Serum fluorescent value for Negative Control bead
NC Serum	Negative Control Serum (OLI Cat. # LS-NC) validated for a given lot of LABScreen beads

2. For LABScreen PRA or LABScreen Single Antigen:

$$\text{NBG ratio} = \frac{\text{S\#N / SNC bead}}{\text{BG\#N / BGNC bead}}$$

For LABScreen Mixed:

$$\text{NBG ratio} = \frac{\text{S\#N - SNC bead}}{\text{BG\#N - BGNC bead}}$$

Note: If (BG#N-BGNC bead) <50 then use 50 as a default threshold value.

D. Determination of Positive/Negative Cut-Off

1. For LABScreen PRA and LABScreen Mixed:
   a. Select the NBG ratio that gives a significant shift over background fluorescent value when the background value is obtained using the negative control serum in 3 - 5 replicate tests. If you prefer, test 5 - 10 serum samples from non-transfused, non-transplanted male donors to obtain an average background value.
   b. Validate the cut-off using 5 - 10 reference alloserum samples with defined HLA antibody specificity. The NGB ratio values for expected positive antigen reactions should be above the cut-off.
   c. Additional positive/negative reactions may be noted. If necessary, adjust the LABScreen assay cut-off to match the sensitivity of a previously accepted antibody detection assay.
   d. For high PRA serum, the patient's own antigen(s) may show weak positive reactions. For such cases, the fluorescence value for the patient's own antigen may be used as the cut-off.
2. For LABScreen Single Antigen:
   a. Test negative control serum or several negative serum samples (see 1a, above).
   b. Define working range:

      Working Range = NBG ratio maximum - NBG ratio minimum
   c. Define cut-off points within the working range:

      Relative NBG ratio cut-off = X% (working range) + NBG ratio minimum, where X% = user-defined percent cut-off point within the working range for negative (1), gray area(2), weak positive (4) and strong positive (8).
   d. Set criteria to define positive vs. negative reactions, for example:
      (1) If [NBG ratio max/NBG ratio min] >8, apply the calculation in 2c.
      (2) If [NBG ratio max/NBG ratio min] <8 AND
         (a) NBG ratio max >5, then NBG ratio min should be adjusted to one half of the NBG ratio max and the relative NBG ratio cut-off should be re-computed (as in 2c) based on the adjusted NBG ratio min. The reaction is then scored as above.
         (b) NBG ratio max <5, then the reaction of the test serum with that bead is negative. Assign a score of "1".
   e. Test several reference allosera as in 1b above, using the relative NBG ratio to validate the cut-off.
      (1) Establish a strong and weak reactivity cut-off based on the performance of the reference allosera, relative to an established assay.
      (2) It may be helpful to plot the NBG ratio values in a histogram for visualization of the HLA reactivity pattern of each serum.
3. Higher or lower sensitivities can be obtained by adjusting the cut-off.
4. Optional analysis – HLA Fusion™ software.

LIMITATIONS OF THE PROCEDURE

- Sera or plasma samples that contain contaminants or aggregates may clog the LABScan flow analyzer and generate inaccurate data. Aggregates in the test specimen should be removed by centrifugation or filtering the serum prior to testing.
- The presence of IgG-IgM immune complex may cause inhibition in some patient samples. Samples should be treated to reduce this presence according to the protocols determined by the laboratory, however, samples

should not be heat treated as they may cause non-specific background – please reference bibliography section for more information.[7,8,9,10, 11]

- Ambient temperature may affect LABScan 100 and LABScan3D performance. If the ambient temperature changes, the machine may need to be re-calibrated. Consult the manufacturer's manual for more information.
- The LABScan 100 and LABScan3D flow analyzer must be properly calibrated and maintained. If insufficiently flushed, aggregates of the sample may cause the machine to clog and generate invalid data.
- Assignment of antibody specificity is limited to the HLA antigens presented in each bead panel (see lot-specific worksheet).
- The bead region used for each antigen and the antigen composition of the panel may change from lot-to-lot of product (see the lot specific worksheet).
- Because of the complexity of the HLA allelic definitions, a certified HLA technician or specialist should review and interpret the data, and assign the HLA typing.
- This test must not be used as the sole basis for making a clinical decision.

EXPECTED VALUES

A. LABScreen PRA Class I or Class II

- The reactivity strength of a test serum to each bead can be compared to distinguish the strong positive, weak positive and negative reactions. Reactivity ratios can be ranked within different ranges, if a scoring system is desired.
- Our data show NBG ratios > 1.5 in the LABScreen PRA test (using the LABScan 100) correlate well with positive reactions in the FlowPRA test.
- For calculation of percent PRA (Panel Reactive Antibody), divide the number of positive reactions by the number of valid reactions for that test serum.
- To determine the specificity of HLA antibody, enter the reaction score into the lot specific Worksheet to analyze the reaction pattern.

B. LABScreen Mixed

- Score HLA Class I and Class II reactions separately, according to reactivity strength of the serum for each bead set.
- If anyone bead in the mixed assay is positive, then the result should be assigned as positive.
- Our data show that NBG ratios >2.2 in the LABScreen Mixed test (using the LABScan 100) correlate well with positive reactions in LAT™ Mixed.

C. LABScreen Single Antigen

- Allosera may produce signal/background ratios that are much higher than those obtained in the PRA assay. Establishing the assay cut-off(s) using the relative NBG ratio is one way of normalizing the data (see Results, Section D-2c).
- Our data show that a positive/negative cut-off or relative NBG ratio >15% of the working range NBG ratio calculated for each test serum (using the LABScan 100) will give results comparable to the LABScreen PRA assay.

D. General Guidelines

- Each bead count should be over 50. A lower bead count may be due to sample loss during the wash steps. It could also be due to improper calibration or clogging of the LABScan 100 or LABScan3D flow analyzer, or by photo-bleached beads that dropped out from the mapped region.
- Signal values are the fluorescence intensity of each bead set vs. the test serum. A negative control serum should be tested with the same batch of samples to establish the background value(s) for that test run.

- Negative Control Serum (OLI Cat. # LS-NC or equivalent) is recommended. Using any other negative control serum may require adjustment of cut-off values.
- Negative Control Beads (Ag ID = NC) are not coated with HLA antigen. The fluorescence value may vary among different sera due to non-specific binding of the sera or to insufficient washing. The NC value is usually less than 500 except for serum samples with a high background. It should always be lower than 1500 and less than or equal to half of the PC value.
- Positive Control Beads are coated with purified human IgG, which should bind to the secondary antibody to produce a positive signal. The PC value should be over 500 and at least twice the NC value.

E. Validation of the Assay

- The cut-off value of signal to background should be validated if a new negative control serum is used.
- For a given serum, the value for PC/NC should be greater than 2. A lower value may be due to an extremely high NC bead background value for the test serum, a high HLA bead signal for the NS control, or a low signal from the secondary antibody or the LABScan 100 and LABScan3D flow analyzer. In this case, the data may have to be confirmed.
- Each user should evaluate the performance of the assay in their laboratory to validate the cut-off value(s) selected.
- Plasma samples may give lower FI or higher background values than serum. The user may wish to normalize the data if comparing results between sera and plasma samples (see Reference 5) for the same or different test subjects.

SPECIFIC PERFORMANCE CHARACTERISTICS

A. Using the assay cut-offs referenced under Expected Values, above, LABScreen assays have given results comparable to the results of the One Lambda FlowPRA® and LAT™ assays. However, HLA antibody patterns may be quite complex. A given test sample may contain several HLA Class I and Class II antibody specificities, each with a different avidity; however, not all specificities will be recognized in assays with lesser sensitivity. Therefore, each laboratory should establish and validate the assay cut-offs for their own use based on their expertise in recognizing HLA CREG patterns and an evaluation of the assay performance using HLA allosera with defined specificities.

B. Comparison of serum vs. plasma for 1,000 blood donors in the NIHLBI REDS-II study (5) showed good correlation within the working range of the assay. For anti-HLA CI and CII antibodies the R2 values were 0.88 and 0.91, respectively. However, the NBG ratio was generally 1.3-fold higher for serum samples.

C. If high background is seen, this may indicate improper washing during the test protocol. High negative control background may cause inaccurate normalized MFI values.

D. Clinical performance testing was conducted for LABScreen products at three different clinical sites, using 240 random samples – See Table A. Clinical Performance.

E. Clinical reproducibility testing was conducted for LABScreen products at three different clinical sites using 16 (LS1PRA, LS2PRA, LS1A04) and 32 (LSM12) samples, consisting of 10 runs each, in triplicate – see Table B. Clinical Reproducibility.

F. Clinical testing used a default cut off value, with scores of >4 were considered positive.

Table A - Clinical Performance

LSM12

		LABScan 3D +	LABScan 3D -
LABScan 100	+	573	11
	-	4	119
		Undefined	40
		Total Defined	707

	Positive Agreement	Negative Agreement	Overall Agreement (excluding undefined)	Overall Agreement (including undefined)
Point estimate	98%	97%	98%	93%
One-sided 95% lower confidence limit	97%	93%	97%	91%

LS2PRA

		LABScan 3D +	LABScan 3D -
LABScan 100	+	939	57
	-	187	7781
		Total	8964

	Positive Agreement	Negative Agreement	Overall Agreement
Point estimate	94%	98%	97%
One-sided 95% lower confidence limit	93%	97%	97%

LS1PRA

		LABScan 3D +	LABScan 3D -
	+	2060	260
	-	446	16905
		Total	19671

	Positive Agreement	Negative Agreement	Overall Agreement
Point estimate	89%	97%	96%
One-sided 95% lower confidence limit	88%	96%	96%

LS1A04

		LABScan 3D +	LABScan 3D -
	+	3245	214
	-	682	12062
		Total	16203

	Positive Agreement	Negative Agreement	Overall Agreement
Point estimate	94%	95%	94%
One-sided 95% lower confidence limit	93%	94%	94%

Table B - Clinical Reproducibility

LSM12	Overall Agreement (excluding undefined)	Overall Agreement (including undefined)
Point estimate	98%	93%
One-sided 95% lower confidence limit	97%	93%

LS1PRA	Overall Agreement	LS2PRA	Overall Agreement	LS1A04	Overall Agreement
Point estimate	99%	**Point estimate**	99%	**Point estimate**	98%
One-sided 95% lower confidence limit	99%	**One-sided 95% lower confidence limit**	99%	**One-sided 95% lower confidence limit**	98%

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TRADEMARKS AND DISCLAIMERS

All One Lambda products are designed to assist personnel experienced in HLA analysis by suggesting typing results or antibody assignments. All test results must be carefully reviewed by such qualified personnel to assure correctness.

EUROPEAN AUTHORIZED REPRESENTATIVE

EC REP MDSS GmbH, Schiffgraben 41, 30175, Hannover, Germany

EXPLANATION OF SYMBOLS

Symbol	Description
REF	Catalog number
IVD	In vitro diagnostic medical device
	Consult instructions for use
	Caution, consult accompanying documents
	Biological risks
	Temperature limitation
	Manufacturer
EC REP	Authorized representative in the European Community

REVISION HISTORY

Revision	Date	Revision Description
02	08/14/2019	Modify xPONENT software designation to encompass current and future version of the software
03	09/20/2019	Updated contact information and address to reflect change in legal manufacture site.
04	Current	LABScreen PI updated to include information for LABScreen Single Antigen ExPlex Class I and Class II (LS1AEX01 and LS2AEX01). Clarified the sealing step in Section II, Step 2. Updated the Section IV table to include device specifications.

CE 0197 CE

*0197 Applies to Annex II List B products only.

LABType™ SSO TYPING TESTS

REF

Catalog ID	Product Name
RSSO1A*	LABType™ SSO Class I A Locus Typing Test
RSSO1B*	LABType™ SSO Class I B Locus Typing Test
RSSO1S4*	LABType™ SSO Class I Bw4 Supplement Typing Test
RSSO1S1*	LABType™ SSO Class I B7 Supplement Typing Test
RSSO1E47*	LABType™ SSO Class I Exon 4-7 Supplement Typing Test
RSSO1C	LABType™ SSO Class I C Locus Typing Test
RSSO2P	LABType™ SSO Class II DPA1/DPB1 Typing Test
RSSO2Q	LABType™ SSO Class II DQA1/DQB1 Typing Test
RSSO2B1*	LABType™ SSO Class II DRB1 Typing Test
RSSO2345*	LABType™ SSO Class II DRB3,4,5 Typing Test
RSSOH1A*	LABType™ HD Class I A Locus Typing Test
RSSOH1B*	LABType™ HD Class I B Locus Typing Test
RSSOH1C	LABType™ HD Class I C Locus Typing Test
RSSOH2B1*	LABType™ HD Class II DRB1 Typing Test
RSSOMICA	LABType™ SSO MICA

Catalog ID	Product Name
RSO1AT*	LABType™ SSO Class I A Locus Typing Test - 20 tests
RSO1BT*	LABType™ SSO Class I B Locus Typing Test - 20 tests
RSO1S4T*	LABType™ SSO Class I Bw4 Supplement Typing Test - 20 tests
RSO1S1T*	LABType™ SSO Class I B7 Supplement Typing Test - 20 tests
RSO1E47T*	LABType™ SSO Class I Exon 4-7 Supplement Typing Test - 20 tests
RSO1CT	LABType™ SSO Class I C Locus Typing Test - 20 tests
RSO2PT	LABType™ SSO Class II DPA1/DPB1 Typing Test - 20 tests
RSO2QT	LABType™ SSO Class II DQA1/DQB1 Typing Test - 20 tests
RSO2B1T*	LABType™ SSO Class II DRB1 Typing Test - 20 tests
RSO2345T*	LABType™ SSO Class II DRB3,4,5 Typing Test - 20 tests
RSOH1AT*	LABType™ HD Class I A Locus Typing Test - 20 tests
RSOH1BT*	LABType™ HD Class I B Locus Typing Test - 20 tests
RSOH1CT	LABType™ HD Class I C Locus Typing Test - 20 tests
RSOH2B1T*	LABType™ HD Class II DRB1 Typing Test - 20 tests

IVD For In Vitro Diagnostic Use.

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INTENDED USE

DNA typing of HLA Class I or Class II alleles

SUMMARY AND EXPLANATION

Historically, the established method for the determination of HLA antigens has been the lymphocytotoxicity test.[1] However, with the advent of PCR technologies, DNA based tissue typing techniques have become routine in the laboratory. For most DNA-based methodologies, the PCR process is used only as an amplification step to acquire the needed target DNA. The HLA typing process then requires a post-amplification step to discriminate between the different alleles (e.g., RFLP, SSOP, reverse dot blot). LABType™ SSO uses sequence-specific oligonucleotide probes (SSO) bound to fluorescently coded microspheres to identify alleles encoded by the sample DNA. The introduction of a step to amplify the target DNA by polymerase chain reaction (PCR), coupled with hybridization and detection in a single reaction mixture, makes this method suitable for both small and large-scale testing. In contrast to the lymphocytotoxicity reaction scale (1 = negative to 8 = positive), LABType™ test results are either positive or negative. This abolishes the need for complicated interpretation of results. In addition, single nucleotide changes can be discriminatory in PCR-SSO, while cross-reacting groups (CREGs) provide major challenges to serological typing.

PRINCIPLES

LABType™ applies Luminex® technology to the reverse SSO DNA typing method. First, target DNA is PCR-amplified using a group-specific primer. The PCR product is biotinylated, which allows it to be detected using R-Phycoerythrin-conjugated Streptavidin (SAPE).

The PCR product is denatured and allowed to rehybridize to complementary DNA probes conjugated to fluorescently coded microspheres. A flow analyzer, either the LABScan™ 100 (Luminex® 100/200) or LABScan3D™ (Luminex® FLEXMAP 3D), identifies the fluorescent intensity of PE (phycoerythrin) on each microsphere. The assignment of the HLA typing is based on the reaction pattern compared to patterns associated with published HLA gene sequences.

REAGENTS

A. Identification

The LABType™ SSO DNA typing system provides sequence-specific oligonucleotide probes immobilized on microspheres for identification of HLA alleles in amplified genomic DNA samples through a controlled DNA-DNA hybridization reaction, followed by flow analysis using either the LABScan™ 100 or LABScan3D™ flow analyzer. The system components consist of:

- Pre-optimized and tested mixture of microspheres with probes covalently attached
- Hybridization reaction buffers to facilitate the binding of target DNA to the probe
- Wash Buffer to wash off unbound DNA
- SAPE buffer for diluting Stock SAPE solution
- DNA amplification reagents (pre-optimized HLA loci-specific primer mix): With every LABType product, the use of the locus-specific primer mix and bead mix is essential. These reagents are lot specific and are not interchangeable between lots.
- D-mix (specially formulated amplification buffer mix).

The microsphere mixture consists of a set of fluorescently labeled microspheres that bear unique sequence-specific oligonucleotide probes for HLA alleles. Each microsphere mixture includes negative and positive control microspheres for subtraction of non-specific background signals and normalization of raw data to adjust for possible variation in sample quantity and reaction efficiency. The microsphere mixtures are pre-optimized for particular PCR products obtained by DNA amplification using the specified HLA locus-specific primer mixes. The HLA locus-specific primer mixes are pre-optimized for amplification of specific HLA genes from 40 ng of purified genomic DNA in 20 µl volume when used in conjunction with D-mix, the prescribed amount of recombinant Taq polymerase, and the PCR reaction profile detailed below. For each lot, see provided worksheet for the specific HLA alleles that can be identified by each probe using the procedures described below. For lot specific probe sites, refer to the *Bead Probe Information* document.

B. Warning or Caution

1. FDA Designation: IVD
2. **Warning**: Ethidium bromide, which is used for gel staining and which is not included with this product, is a known carcinogen. Handle with appropriate caution. Can be harmful if absorbed through skin. Avoid splashing in eyes or on skin or clothing. Keep tightly sealed. Wash thoroughly after handling. Flush spill area with water spray.
3. **Warning**: Denaturation Buffer and Neutralization Buffer are corrosive and may cause burns. In case of contact, immediately flush eyes or skin with a copious amount of water for at least 15 minutes while also removing contaminated clothing and shoes (see MSDS).
4. **Caution**: LABType™ SSO Bead Mixture is light sensitive and must be protected from light.
5. **Caution**: Use LABType™ SSO Bead Mixture within three months after it is thawed.
6. Refer to the Material Safety Data Sheet for detailed information.

C. Preparing Reagents for Use

See *Directions for Use* in this document.

D. Storage Instructions

All of the LABType™ SSO Typing Tests can be safely stored frozen at -80° to -20°C in the product box. Avoid unnecessary handling. It is recommended to keep the entire package intact and frozen upon receipt until ready to use. See Table below for individual component storage conditions.

Component	Storage Conditions
LABType SSO Bead Mixture	-80˚C to -20˚C *Protect from light* *After thawing store at 2˚C to 8˚C for 3 months* *Do not refreeze and thaw*
Locus-Specific Primer Set	-80˚C to -20˚C *May repeat freeze-thaw; store frozen*
Denaturation Buffer	-80˚C to 25˚C
Neutralization Buffer	-80˚C to 25˚C
Hybridization Buffer	-80˚C to 25˚C
Wash Buffer	-80˚C to 25˚C
SAPE Buffer	-80˚C to -20˚C *After thawing store at 2˚C to 8˚C for 3 months*
Primer Set D-mix	-80˚C to -20˚C *May repeat freeze-thaw; store frozen*

E. Instability Indications

1. Beads that exhibit discoloration, or aggregation that cannot be removed by vortexing, should be considered unusable.
2. If salts have precipitated out of any of the product reagents during shipping or storage, re-dissolve by extended vortexing at room temperature (20° to 25°C).
3. D-mix aliquots, upon thawing at room temperature (20° to 25°C), should be pink to light purple in color. Any D-mix aliquot without the specified coloration should be considered unusable.

INSTRUMENT REQUIREMENTS

- LABScan™ 100 (Luminex 100/200) or LABScan3D™ (Luminex® FLEXMAP 3D®) flow analyzer
- Luminex® XY Platform (optional accessory for automated 96-sample reading on the LABScan™ 100 flow analyzer from Luminex Corporation)

- Centrifuge
- Rotor for 1.5 ml microfuge tube (14,000 to 18,000 g)
- Swing bucket rotor for 96-well microplate (1000 - 1300 g)
- Vortex mixer with adjustable speed

- Thermocycler - Veriti™ 96-Well Thermal Cycler or Thermocycler
  - Block format 0.2 mL alloy
  - Features Standard 0.2 mL 96-well format
  - Heated lid capable of maintaining 103°C
  - Max block ramp rate 3.90°C/sec
  - Max sample ramp rate 3.35°C/sec
  - Enabled to run 9600 emulation mode at sample ramp rate of +0.8°C/sec and -1.6°C/sec
  - Maximum temperature differential 25°C across whole block, 5°C zone-to-zone
  - Temperature accuracy ±0.25°C (35–99.9°C) zone
  - Temperature range 4.0°C to 99.9°C zone
  - Temperature uniformity <0.5°C (20 sec after reaching 95°C) zone
  - PCR volume range 10–80 µL zone

SPECIMEN COLLECTION AND PREPARATION

A. DNA can be purified from sample sources including whole human blood, isolated lymphocyte cells (buffy coat) from blood, blood on filter paper, lymph nodes, buccal swabs, and bone marrow with validated method that meets the criteria below. The DNA sample to be used for PCR should be re-suspended in sterile water or in 10 mM Tris-HCl, pH 8.0 – 9.0 at an optimal concentration of 20 ng/µl with the A260/A280 ratio of 1.65 - 1.80. Other specifications used should be validated by the laboratory.

B. Samples should be free from any inhibitors of DNA polymerase, and should not be re-suspended in solutions containing chelating agents, such as EDTA, above 0.5 mM in concentration.

C. DNA samples may be used immediately after isolation or stored at -20°C or below for extended periods of time with no adverse effects on results.

D. DNA samples should be shipped at 4°C or below to preserve their integrity during transport.

PROCEDURE

A. Materials Provided

***NOTE*:** *The volumes provided are slightly more than the amount required for testing. This is to account for inadvertent losses that may result from pipetting. Do not mix components from different lots of products.*

100 Tests per Package		**20 Tests per Package**	
2.25 ml Denaturation Buffer - 1 vial	4.95 ml SAPE Buffer - 1 vial	2.25ml Denaturation Buffer - 1 vial	990 µl SAPE Buffer - 1 vial
2.5 ml Neutralization Buffer - 1 vial	1.38 ml Primer Set D-Mix - 2 vials of 690 µl each	100 µl Neutralization Buffer - 1 vial	276 µl Primer Set D-Mix - 1 vial
3.4 ml Hybridization Buffer - 1 vial	400 µl Locus-Specific Primer Set - 1 vial	680µl Hybridization Buffer - 1 vial	80 µl Locus-Specific Primer Set - 1 vial
55 ml Wash Buffer - 1 bottle	Bead Mixture – 400 µl LABType™ SSO primary -1 vial * 20 µl Supplement – 1 vial*	10 ml Wash Buffer - 1 vial	80 µl LABType™ SSO or HD Bead Mixture - 1 vial

**** NOTE:** LABType™ (100 test) kits may contain two bead vials as needed for continued optimum resolutions: primary bead mix and a supplement bead mix.*

B. Materials Required, But Not Provided

1. Deionized water
2. 70% ethanol
3. 20% chlorine bleach
4. R-Phycoerythrin-Conjugated Streptavidin--SAPE
5. Sheath fluid (OLI Cat.#LXSF20 or LSXF20X5)
6. Recombinant Taq polymerase (OLI catalog IDs TAQ30, TAQ50 and TAQ75)
7. 15 - 50 ml disposable tubes
8. 96-well, thin-walled PCR tray, or tubes, and holder that can withstand 1000 – 1300 g in a centrifuge

   Caution: *PCR plate must have tight contact with heating block.*

9. Tray seal

   NOTE: *PCR trays (25) and tray seals (180) sufficient for 2400 samples can be ordered from One Lambda (OLI Cat. #PCRTRAC)*

10. Electrophoresis apparatus/power supply—150V minimum capacity UV transilluminator (Fotodyne FOTO/UV®21 or equivalent)
11. Photographic or image documentation system
12. Electrophoresis running buffer – example: 1x TBE buffer (89mM Tris-borate; 2 mM disodium EDTA, pH 8.0) with 0.5 μg/ml ethidium bromide or 5XTBE Buffer with ethidium bromide
13. Electrophoresis grade agarose (e.g., FMC Seakem® LE or equivalent)
14. PCR Pad
15. Crushed ice bath or equivalent.

C. Directions for Use

Caution: *Special care must be taken in the aliquoting process. Failure to follow the steps described below may result in reagent loss.*

1. Bead Handling and Storage

a. Use of the recommended disposables (tubes, trays, and tips) can minimize loss of beads due to non-specific adhesion. (See "Material Required, but Not Provided.")

b. LABType™ SSO beads can settle and aggregate if left in a tube. Beads must be evenly distributed before dispensing. Always mix beads vigorously by pipetting several times or by vortexing in horizontal position for 10 to 30 seconds, or as much as necessary, to obtain fully homogeneous mixture.

c. For LABType™ SSO HD products, we recommend the following procedures to help prevent bead aggregation. Immediately after removal of supernatant in step 2f, 2g, and 3c in Test Procedure below, remove as much liquid as possible by inverting and very gently tapping tray on dry paper towel. Place a seal on tray and vortex thoroughly at low speed to loosen the pellets. Proceed to next step as described.

d. LABType™ SSO beads are packaged in an aluminum foil bag. Do not remove beads from foil bag until ready to use.

e. LABType™ SSO beads contain internal fluorescent dye, as well as HLA allele-specific probes, attached to their surfaces. To avoid photo bleaching of the beads, protect beads from light during usage and storage. Store beads at -20ºC in the tightly capped tube provided until ready to use. Cover beads with aluminum foil or equivalent during assay.

   Caution:

   - *Once beads are thawed, store beads at 2° to 8ºC and use within 3 months. Do not refreeze beads.*
   - *Open bags containing Amplification Primer Mixture and D-Mix only in pre-amplication area. Store these items at* -80° to -20° C *in the pre-amplification area.*

2. Amplification (Set up in pre-amplification area.)

a. Enter the "LABType™ PCR Program," into your thermal cycler as shown in **Table 2**. Confirm all parameters.

b. Turn on the thermal cycler to warm up heated lid.

c. Thaw DNA, Amplification Primers, and D-Mix. Keep on ice until use.

d. Adjust the concentration of genomic DNA to 20 ng/μl using sterile water.

e. Vortex D-mix and Amplification Primer for 15 seconds; centrifuge for 3-5 seconds.

f. Using Table 1 below, mix indicated volume of D-mix and Primers. Vortex for 15 seconds, and place on ice. For accurate pipetting of Taq polymerase, it is recommended that you prepare master mix for at least 10 reactions.
g. Add Taq polymerase immediately before use.

Table 1: Amplification Mixture

# of Reactions	D-mix (µl)	Amplification Primer (µl)	Taq Polymerase (µl)
1	13.8	4	0.2
10	138.0	40	2.0
50	690.0	200	10.0
96‡	1491.0	432	21.6 (22)

‡*calculation is generated for 108 samples to prevent shortage*

h. Pipette 2 µl of DNA (at 20 ng/µl) into the bottom of a tube (for final volume of 20 µl per PCR reaction). Store the tubes or tray partially covered to prevent evaporation and contamination.
i. Add an appropriate amount of Taq polymerase (e.g., 0.2µl (typically at 5 U/ul) per 20 µl reaction) to the Amplification Mixture prepared in Step 2.f.
j. Vortex for a few seconds, and centrifuge for 3-5 seconds.
k. Aliquot 18 µl of Amplification Mixture into each well containing DNA.

Caution: *To prevent cross-contamination, be sure not to touch the pre-aliquoted DNA at the bottom.*

l. Cap or seal. If you are using a tray seal, make sure it is pressed tightly against the rim of each well. Place a PCR Pad appropriate for the thermal cycler on the tray before closing the lid. Close and tighten the lid of the thermal cycler.
m. Run "LABType™ SSO PCR Program," shown in Table 2.
n. For Verti™ 96-Well Thermal Cycler, set "ramp speed" to the 9600 program. For other systems, consult the manufacturer's documentation to adjust ramp speed to the specifications outlined in Instrument Requirements Use of a significantly different ramp speed will affect amplification efficiency and final results.

Table 2: LABType™ SSO PCR Program

Step	Temperature and Incubation Time	# of Cycles
Step1:	96ºC 03:00	1
Step 2:	96ºC 00:20	5
	60ºC 00:20	
	72ºC 00:20	
Step 3:	96ºC 00:10	30
	60ºC 00:15	
	72ºC 00:20	
Step 4:	72ºC 10:00	1
Step 5:	4ºC forever	1

o. Amplified DNA is now ready to be tested using the Test Procedure in section D.

NOTE: *It is recommended to first use 2 - 5 µl of amplified DNA for analysis by gel electrophoresis. Confirmation of an amplification product (band) prior to hybridization assay ensures generation of optimal signals.*

p. If the amplified product is not used immediately, store covered DNA tray at -80° to -20° C for up to one month.

3. Test Set-Up

a. Turn on the LABScan™ 100 and XY Platform or LABScan3D™ and follow the start-up procedure described in Section D of the Directions for Use. The LABScan™ 100 or LABScan3D™ requires at least 30 minutes to warm up.
b. Turn on thermal cycler and run program to 60°C HOLD, or equivalent, for at least 1.5 hours (or hold forever). Have a PCR Pad appropriate for your thermal cycler ready for use. Be sure to wait until the heated lid of the thermal cycler reaches the appropriate temperature before use. Use the appropriate 96-well PCR tray holder to ensure the proper incubation temperature.
c. Remove all reagents (except brown 100X SAPE bottle) from storage to room temperature. Aliquot necessary volumes of reagents into clean containers. (Use the tables below for reference). Be sure to prepare 1X SAPE during the third wash step. Remove the 100X SAPE bottle from storage only when needed, and return immediately to 2° to 8° C. Return any unused portions of the Bead Mixture to 2° to 8° C.
Caution: *Do not refreeze Bead Mixture after thawing.*

4. LABType™ Bead Preparation (for 100-Test Kit containing two bead vials):

a. For LABType™ kits containing 2 bead vials, give tubes a quick spin (10-15 seconds at 100 RCF (relative centrifugal force) in most small centrifuges) immediately after thawing.
b. Vortex vials at medium strength for 20 seconds, then give a quick spin again as described above.
c. Take the primary bead vial and slowly, but thoroughly, pipette up and down several times using P1000 or equivalent to mix bead solution and to prime the pipette tip.
d. Using the same pipette tip used in Step (c), carefully transfer the entire volume of the primary beads into the supplemental bead vial.
e. Discard the empty primary bead vial. The supplemental tube is labeled with the new lot/batch identifier for the combined beads. This lot number is associated with the correct analysis cat files and data sheets.
f. Mix the combined beads vigorously by vortexing the capped tube 3 times for 10 seconds each to obtain a homogenous bead mixture. Use immediately or store at condition described in page 2, Storage Instructions. Be sure to vortex the bead vial at medium speed for at least 20 seconds immediately before use.

Table 3: Reagent Preparation

Reagent	Amount per Test	Preparation Method and Suggestions
Bead Mixture	4 µl	• Aliquot appropriate volume, plus extra volume*, for the required number of tests into a clean tube at room temperature. • Protect from light. Use the entire contents of the Bead Mixture tube for 96 samples. • Vortex immediately before use.
Hybridization Buffer	34 µl	• Aliquot for exactly the same number of tests as used for the Bead Mixture. • Add to pre-aliquoted Bead Mixture to prepare Hybridization Mixture. • Keep at room temperature (20° to 25° C) until use.
Wash Buffer	480 µl	• Aliquot appropriate volume, plus extra volume*, for the required number of tests, and keep at room temperature (20° to 25° C). • Use the entire contents in a trough for 96 samples.
Denaturation Buffer	2.5 µl	• Aliquot appropriate volume, plus extra volume*, for the number of tests. • Use the entire contents in a trough for 96 samples. Keep at room temperature (20° to 25° C).
Neutralization Buffer	5 µl	• Aliquot appropriate volume, plus extra volume*, for the number of tests. • Use all 2.5 ml for 96 samples. Keep at room temperature (20° to 25° C).
SAPE Stock (100X)	0.5 µl	• During the last centrifugation step, prepare 1X SAPE solution by making 1:100 dilution of SAPE Stock with SAPE Buffer for the appropriate number of tests, plus extra volume.*
SAPE Buffer	49.5 µl	• Protect from light. • Prepare enough 1XSAPE solution for 96 samples (around 110 sample worth depending on

Reagent	Amount per Test	Preparation Method and Suggestions
		observed pipetting error). • Keep SAPE Stock bottle at 2° to 8° C.

NOTE: *The extra volume required depends on pipetting technique and calibration status of equipment. Use a full volume of Bead Mixture in the tube provided (enough for approximately 110 tests) for 96 tests. Prepare 1X SAPE for 115 tests, and use entire volume of other reagents to prevent a shortage. We recommend calibration of all pipetting devices and testing of these devices by aliquoting water. For reagents provided in excess volume, such as Denaturation and Neutralization Buffer, you may use a trough for multichannel pipetting.*

Table 4: Reagent Volumes

Number of Tests	Denaturation Buffer (µl)	Neutralization Buffer (µl)	Hybridization Buffer (µl)	Wash Buffer (µl) Tray Method	Bead Mixture (µl)
1	2.5	5	34	480	4
10	25	50	340	4800	40
20	50	100	680	9600	80
50	125	250	1700	24000	200
96	240	480	3264	46080	384

Table 5: SAPE and SAPE Buffer Volumes

Number of Tests	SAPE Stock Volume (µl)	SAPE Buffer Volume (µl)
1	0.5	49.5
10	5.0	495.0
20	10.0	990.0
50	25.0	2475.0
96	48.0	4752.0

NOTE: *Volume of reagents in Tables 4 and 5 are for the exact number tests. The actual number of aliquots differs depending on pipetting accuracy. For a full 96-sample assay, we recommend using the entire bead mixture, the entire volume of hybridization buffer, 57.5 µl stock SAPE, and 5693 µl of SAPE buffer, which is slightly more than the exact amount required for the test.*

D. Test Procedure

TECHNICAL PRECAUTIONS

1. To assay a small number of samples (48 or fewer) you may use a 96-well tray, a tray that has been cut to the appropriate number of wells, or a 0.2 ml thin-wall PCR strip tube. Be sure to use a tube rack when using a cut-off tray or strip tube.
2. Mixing of samples in a 96-well tray involves sealing of the tray and low speed vortexing for a few seconds. Adjust the speed of the vortex mixer so that liquid inside the 96-well PCR tray is sufficiently agitated without excessive splashing. Note the speed setting, and use it for the 96-well tray method.
3. Sealing of the 96-well PCR tray should be done carefully and completely to prevent well-to-well sample contamination. Seal the tray by pressing the seal against each rim of the 96 wells. Do not re-use tray seals. Use a fresh seal for each step that requires application of a tray seal. A repeater pipette may be used where applicable; however, a repeater pipette is usually less accurate in volume delivery.

4. We recommend regular calibration and a manual volume check for each volume to be delivered. Do not use a repeater pipette for dispensing the Hybridization Mixture.

1. Denaturation/Neutralization

a. Prepare a crushed ice bath.
b. Place a clean 96-well plate in a tray holder.
c. Transfer 2.5 µl Denaturation Buffer into a well of a clean 96-well plate.
d. Add 5 µl of each amplified DNA. Make sure sample location and ID are noted. Mix thoroughly (preferably by pipetting up and down), and incubate at room temperature (20 - 25° C) for 10 minutes.

NOTE: *Amplified DNA can be aliquoted first and subsequent addition of Denaturation Buffer.*

e. Add 5 µl Neutralization Buffer with pipette, and mix thoroughly (preferably by pipetting up and down). Note the color change from bright pink to pale yellow or clear.
f. Place PCR plate with neutralized PCR product on the ice bath.

Caution: *Avoid contamination of PCR product with water.*

2. Hybridization

NOTE: *Make sure that the thermal cycler has been turned on and the 60°C program has been started to warm the thermal block.*

a. Combine appropriate volumes of Bead Mixture and Hybridization Buffer to prepare Hybridization Mixture.
b. Add 38 µl Hybridization Mixture to each well.
c. Cover tray with tray seal and vortex thoroughly at low speed.
d. Remove from tray holder and place PCR tray into the pre-warmed thermal cycler (60° C).
e. Place PCR Pad on top of tray or caps on PCR tubes. Close and tighten lid. Incubate for 15 minutes.
f. Place tray in tray holder and remove tray seal. Quickly add 100 µl Wash Buffer to each well. Cover tray with tray seal. Centrifuge tray for 5 minutes at 1000 -1300 g. Place tray in tray holder and remove wash buffer.
g. Repeat step 2.f above two more times for a total of three wash steps. Remember to prepare 1X SAPE solution during third centrifugation.

3. Labeling

a. Place tray in tray holder. Add 50 µl of 1X SAPE solution to each well. Place tray seal on tray and vortex thoroughly at low speed. Place tray in the pre-heated thermal cycler (60° C). Place PCR Pad on top of tray or caps on PCR tubes. Close and tighten lid. Incubate for 5 minutes.
b. Remove tray. Place tray in tray holder. Remove seal and quickly add 100 µl Wash Buffer to each well.
c. Cover tray with tray seal. Centrifuge tray for 5 minutes at 1000 – 1300 g. Place tray in tray holder and remove supernatant.
d. Add 70 µl Wash Buffer to each well. Gently mix by pipetting. Transfer to reading plate using an 8- or 12-channel pipet. Avoid sample- to-sample contamination by using fresh pipette tips.
e. NOTE: Final volume should be at least 80 µl.
f. Cover tray with tray seal and aluminum foil. Keep tray in the dark and at 4°C until placed in the LABScan™ 100 or LABScan3D™ for reading.
g. For the best results, read samples as soon as possible. Prolonged storage of samples (more than 4 hours) may result in loss of signal. Store samples overnight at 4°C in the dark with a tray seal, if they cannot be read immediately. Be sure to thoroughly mix the samples immediately before reading.

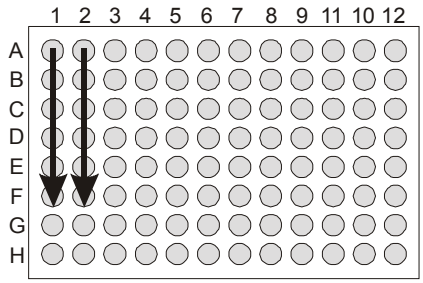

Figure 1 The Luminex® XY Platform reads the sample in the following pattern: A1 to H1, A2 to H2, A12 to H12.

E. Data Acquisition

NOTE: *Described below is a general guide to data acquisition. Details on the use of the LABScan™ 100 or LABScan3D™, may be found in the Luminex® 100/200[3] or Luminex® FLEXMAP 3D® User's Manual[4].*

1. Turn on the system and set up the LABScan™ 100 and/or LABScan3D™ for sample acquisition and calibration according to the Luminex User's Manual for the software version currently being used.
2. Choose a template/protocol according to the product catalog ID and lot number.
   a. Acquisition templates/protocols are available from One Lambda on a CD or are downloadable via the One Lambda website.
   b. To create your own acquisition template, follow the instructions in the Acquisition chapter of the" Luminex User's Manual." Start Up
3. Create a file name for the samples to be run.
4. Make sure all the template/protocol settings are correct.
5. Enter the sample IDs.

   Caution: *If the same sample is tested more than once, a different ID should be assigned.*
6. The plate is now ready to run.
7. Load the plate onto the XY platform and fill the reservoir with sheath fluid.
8. Click on the START button to initiate the session. After the samples have been run, the data output should be saved in a .csv file.
9. Wash the machine 2 times with sheath fluid at the end of the session.

   NOTE: *Luminex® software versions - LABScan 100 (xPONENT® 3.1* or or higher); LABScan 3D (xPONENT 4.2 or higher) must be used. For LABType HD Analysis - Be sure to designate the new (supplemental) lot/ batch when reading and analyzing data. Capture and save the entire run file from the Luminex® flow analyzer for data analysis.*

RESULTS

A. Data Calculation

1. The mean fluorescence intensity (MFI) generated by the Luminex® Data Collector software, or equivalent, contains the FI for each bead (or probe bound to the bead) per sample. The percent positive value is calculated as:

   Percent Positive Value = 100 x [MFI (Probe n) - MFI (Probe Negative Control)/ MFI (Probe Positive Control) - MFI (Probe Negative Control)]

   The positive reaction is defined by the percent of positive values for the probe higher than the pre-set cut-off value for the probe. The negative reaction is defined as the percent of positive values lower than the cut-off value. Under the controlled product QC environment, the MFI for negative control is typically 0-100 and can vary between lots and locus-specific products. Signals outside of the range may represent inefficient controls

of the assay parameters such as sample quantity and/or quality of sample, technique, instrument calibration, and state of all reagents including amplified DNA, buffers, SAPE and the bead mixture.

2. Compare calculated percent positive values to the pre-determined cut-off values for each test probe. Assign a positive attribute to probes that have a percent positive above the cut-off and a negative attribute to those below the cut-off. The MFI of the positive control should be within 1200 - 7000 MFI. (The MFI value may fall outside of this range [see *Expected Values*, Section C] and varies for each positive control probe and lot.) The MFI of each probe is normalized against the positive control MFI and is expressed as a percentage of the positive control MFI. The pre-set cut-off value for each probe was established using a 100- to 200-sample DNA panel.

B. Data Analysis

1. Determine HLA allele (or allele groups) of the sample by matching the pattern of positive and negative bead IDs with the information in the LABType™ SSO worksheet or using HLA Fusion™ Software.

   Note: *For LABType™ High Definition assays and LABType™ assays containing a supplemental bead vials it is necessary to use HLA Fusion™ software version 2.0 or higher for data analysis.*

LIMITATIONS OF THE PROCEDURE

The LABType™ SSO system combines an HLA locus-specific DNA amplification process and DNA-DNA hybridization process. The procedure, as well as the equipment calibration described in this product, must be strictly followed.

DNA amplification is a dynamic process that requires highly controlled conditions to obtain PCR products that are specific to a target segment of HLA gene(s). The procedure provided for the DNA amplification process must be strictly followed. In particular, since sample DNA quantity and quality can significantly affect the amplification reaction, a standardized DNA extraction procedure and spectrophotometric measurement of DNA quantity and quality, followed by gel electrophoretic analysis, are strongly recommended.

In addition, to avoid contamination of initial materials with PCR products, all materials generated after DNA amplification (post-PCR materials, including reaction mixes; all disposable plastics; and equipment, such as pipetting devices and gel electrophoresis devices) must be physically separated from materials used before DNA amplification (pre-PCR materials including all disposable plastics, pipetting devices, sample DNAs, all other reagents used to set up amplification reactions).

Routine wipe testing of pre-amplification work area with validated detection method that is compliant with guidelines provided by concerning regulatory body is recommended.

The DNA-DNA hybridization-based assay using LABType™ SSO is a very temperature-sensitive process. The temperature used for the assay must be checked frequently (calibrated). Strict adherence to the temperatures and incubation times described in this procedure is critical for obtaining optimal results.

LABType™ SSO microspheres are light sensitive and must be protected from light as much as possible. Avoid freezing and thawing to ensure maximum shelf life.

The microsphere mixture provided contains a carefully optimized quantity of microspheres sets bearing HLA allele specific probes. Any alteration of the mixture would significantly affect the accuracy of the assay and would void the results. To minimize a loss of microspheres during the assay, follow the protocol described here and use only recommended pipette tips and tubes. The microsphere mixture provided contains a carefully optimized quantity of microspheres sets bearing HLA allele specific probes. Any alteration of the mixture would significantly affect the accuracy of the assay and would void the results.

When compared to SSP, SSO has more ambiguities because the probes used in SSO can interrogate sample DNA at only one region per test, and SSP can interrogate sample DNA at two regions per test. This is a basic limitation of the SSO method, which is well understood by the HLA professional. As mentioned previously, a list of Resolution Limitations is proved for each lot of the LABType™ SSO Typing Tests to aid in interpretation of the reaction pattern and assignment of HLA typing.

All instruments (e.g., thermal cycler, pipetting devices, LABScan™ 100 or LABScan3D™ and heat block) must be calibrated and/or verified according to the manufacturers' recommendations.

For lot-specific information, refer to the *Bead Probe Information* document.

Because of the complexity of the HLA allelic definitions, a certified HLA technician or specialist should review and interpret the data, and assign the HLA typing.

This test must not be used as the sole basis for making a clinical decision.

EXPECTED VALUES

A. Sample Amplification

1. The HLA locus-specific primer mix provided is expected to yield adequate quantity of amplified DNA. Failure to detect an amplification product by ethidium bromide stained agarose gel electrophoresis voids test results.
2. DNA amplification is subject to contamination by previously amplified DNA. Detection of contamination (by performing a control amplification using water or pre-established DNA wipe test for detection of contaminating amplification products) can void test results.

B. LABScan™ 100 and LABScan3D™ Analyzer

1. The LABScan™ 100 or LABScan3D™ is advanced flow analyzers that requires daily maintenance and calibration and/or verification. Refer to the Luminex® 100/200 or Luminex® FLEXMAP 3D® User Manual for all necessary maintenance operation. Daily maintenance includes routine start-up and shut-down procedures. For best performance, calibrate the instrument as part of the start-up routine. Calibrate the instrument whenever the **Δ Cal Temp** temperature shown on the system monitor panel is more than ± 3ºC for the LABScan 100 or more than ± 5ºC for the LABScan 3D.
2. The instrument must pass a calibration test before LABType™ SSO samples are analyzed.

C. Data Acquisition and Analysis

In order to obtain valid data, two parameters, count and Mean Fluorescence Intensity (MFI), must be monitored for each data acquisition. Count represents the total number of beads that has been analyzed, and the count should be above 100 ±25%. A significant reduction in the count suggests bead loss during sample acquisition or assay and can void test results.

MFI represents a PE signal detected within the counted beads. MFI varies based on reaction outcome. The MFI for the positive control probe could vary from lot to lot, and also due to sample quantity and/or quality, technique, instrument calibration, and state of all reagents including amplified DNA, buffers, SAPE and the bead mixture.

Product QC data information in analysis software presents lot-specific values obtained using DNAs that meet sample requirement (see *Specimen Collection and Preparation*).

Users are strongly advised to determine their own range of the control value using reference sample validation tests for every lot. Significant reduction or elevation in MFI for the positive control probe, accompanied by non-assignable reaction patterns, may suggest inadequate sample quantity and/or quality, poor assay efficiency, or instrument failure, and can void test results.

SPECIFIC PERFORMANCE CHARACTERISTICS

In normal samples and using assay and data acquisition conditions that are within the specifications described in this product insert (e.g., starting genomic DNA concentration of 20 ng/µl and purity, OD260/280 of 1.65 to 1.80, hybridization incubation temperature and washing conditions, and the LABScan™ 100 or LABScan3D™ analyzer performance status), positive and negative reactions are determined by comparing the relative Mean Fluorescence Intensity (MFI) of a sample to its corresponding cut-off value. The cut-off value has been experimentally determined for a given lot of LABType™ SSO product, and the cut-off is used to distinguish between positive and negative signals, based on the HLA genotype of a sample. The results are expected to reflect the presence or absence of certain HLA allele(s), providing a clean-cut typing assignment.

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2. Slater RD, Parham P. Mutually exclusive public epitomes of HLA-A, B, C Molecules. Human Immunology 26: 85-89, 1989.
3. The Luminex® 100 User's Manual, Luminex Corporation, PN 89-00002-00-005 Rev. B.
4. Luminex® FLEXMAP 3D® Hardware User Manual, Luminex Corporation PN 89-00002-00-187.
5. Ng J, Hurley CK, Baxter-Lowe LA, et al. Large-scale oligonucleotide typing for HLA-DRB1/3/4 and HLA-DQB1 is highly accurate, specific, and reliable. Tissue Antigens. 1993; 42: 473-479.
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7. Colinas RJ, Bellisario R et al. Multiplexed genotyping of beta-globin variants from PCR-amplified newborn blood spot DNA by hybridization with allele-specific oligodeoxynucleotides coupled to an array of fluorescent microspheres. Clinical Chemistry 46: 996-998, 2000.

TRADEMARKS AND DISCLAIMERS

The LABType™ typing reagents are manufactured and distributed by One Lambda, Inc., 22801 Roscoe Blvd. West Hills, CA 91304 USA Recombinant Taq polymerase is manufactured by F. Hoffmann-LaRoche.

EUROPEAN AUTHORIZED REPRESENTATIVE

EC REP MDSS GmbH, Schiffgraben 41, 30175, Hannover, Germany

Summary of Protocol for 96-Sample Assay

A. Pre Set-Up

1. Turn on LABScan™ 100 or LABScan™ 3D analyzer, and begin the start-up procedure. Turn on the thermal cycler, and start 60°C incubation program.
2. Prepare crushed ice bath (add small amount of water to allow PCR tray to stand straight on ice)
3. Thaw and vortex D-Mix and DNA.
4. Remove all reagents (except 100x SAPE bottle) from storage temperature and use at room temperature.
5. Thoroughly mix entire volume of Hybridization buffer and entire Bead Mixture in a clean tube; protect from light.

B. Amplification

1. Thaw all amplification reagents, and place on ice.
2. Aliquot 2 µl genomic DNA to each of 96 wells in a PCR tray.
3. Mix 432 µl of Primer Mix, 1491 µl of D-Mix, and 22 µl of Taq polymerase. Vortex well and give a quick spin.
   Note: calculation is generated for 108 samples to prevent shortage
4. Aliquot 18 µl of Amplification Mix from Step 3 into all 96 wells containing DNA.
5. Cap or seal the PCR tray.
6. Run the tray in a PCR oven using the LABType™ SSO PCR program.
7. Remove the PCR tray from the PCR oven, and check the amplified DNA on a 2.5% agarose gel (use 5 µl per well).

C. Denaturation/Neutralization

1. In a clean, thin-walled 96-well PCR tray, aliquot 2.5 µl of Denaturation Buffer per well.
2. Add 5 µl per well of amplified DNA. Note the sample locations in the 96 wells.

NOTE: *Amplified DNA can be aliquoted first and subsequent addition of Denaturation Buffer.*

3. Mix thoroughly until the mixture changes to a bright pink color.
4. Incubate at room temperature (20° to 25º C) for 10 minutes.
5. Add 5 µl per well of Neutralization Buffer.
6. Mix thoroughly until the mixture turns clear or pale yellow.
6. Place tray carefully on the ice bath.

D. Hybridization/Washing

1. Aliquot 38 µl Hybridization Mixture (from A.5.above) per well into all neutralized DNA.
2. Place a seal on the tray and vortex thoroughly at low speed.
3. Incubate the tray in a 96-well block in a 60ºC thermal cycler (use PCR Pad) for 15 minutes.
4. Take out the tray. Add 100 µl of Wash Buffer to each well. Place a new seal on the tray, and spin at 1000 g for 5 minutes.
5. Remove supernatant, leaving approximately 10 µl or less.
6. Repeat Steps D.4 and D.5 two more times for total of 3 washes.
7. During the last centrifugation step, prepare 1X SAPE (57.5 µl Stock and 5693 µl SAPE Buffer) and leave covered at room temperature.

E. Labeling

1. After removal of supernatant from the third wash (D.6 above), add 50 µl 1X SAPE per well.
2. Place a seal carefully on the tray and vortex thoroughly at low speed.
3. Incubate at 60ºC in thermal cycler as above for 5 minutes.
4. Take out the tray, and add 100 µl Wash Buffer to each well. Place a new seal on the tray and spin at 1000 g for
5. 5 minutes.
6. Remove supernatant. Add Wash Buffer to make the final volume 80 µl.
7. Mix by pipetting and transfer all samples to a 96-well microplate for data acquisition.

EXPLANATION OF SYMBOLS

Symbol	Description
REF	Catalog number
IVD	In vitro diagnostic medical device
	Consult instructions for use
	Caution, consult accompanying documents
	Biological risks
	Temperature limitation
CE	CE mark
CE 0197	CE mark of medical quality
	Manufacturer
EC REP	Authorized representative in the European Community

REVISION HISTORY

Revision	Date	Revision Description
02	08/14/2019	Modify xPONENT software designation to encompass current and future versions of the software.
03	09/20/2019	Updated contact information and address to reflect change in legal manufacture site.
04	Curent	Update the address to reflect change in legal manufacture site in the Trademarks and Disclaimers Section.

CE 0197

CE

*0197 Applies to Annex II List B products only

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P R O D U C T I N S E R T

5X Tris-Borate-EDTA (5X TBE) Buffer with Ethidium Bromide

Catalogue # 5XTBE100

STORE REAGENTS AT TEMPERATURE INDICATED ON PACKAGE. USE BEFORE EXPIRATION DATE.

INTENDED USE

This 5X TBE buffer with ethidium bromide (EB) is a gel buffer and electrophoresis running buffer for the separation of double stranded DNA on agarose and polyacrylamide gels.

PRINCIPLE

Hind III fragments of λ DNA are resolved properly on a 1% agarose gel using TBE at working concentrations of 0.5X and 1X for the electrophoresis running and gel buffers.

SUMMARY AND EXPLANATION

For agarose gel electrophoresis, 5X TBE should be diluted to a working concentration of either 1X or 0.5X.[1] After several electrophoresis runs, the TBE buffer should be replaced. This is due to the anode solution becoming alkaline and the cathode solution acidic. This results in lowered DNA mobility.

For polyacrylamide gel electrophoresis: It is recommended that a working concentration of 1X TBE be used to provide adequate buffering capacity.[1]

PRECAUTIONS

EB is a known carcinogen. Handle with appropriate caution. Flush spill areas with water spray. Can be harmful if absorbed through skin. Avoid splashing eyes or on skin or clothing. Keep tightly sealed. Wash thoroughly after handling.

COMPOSITION

The 5X TBE buffer is composed of 0.45 M Tris Borate, 0.01 M EDTA, pH 8.3. This buffer contains 2.5 µg Ethidium Bromide per ml.

STORAGE CONDITIONS

Store at room temperature (20 - 25°C). Use by expiration date on bottle. If a significant precipitate forms, the solution should be discarded.

REFERENCE

1. Sambrook, J, Fritsch, EF, Maniatis, T. *Molecular cloning: a laboratory manual*. 2nd ed. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY, p. 6.7.

DIRECTIONS FOR USE:

Dilute, in this container, 5X stock with 400 ml of distilled H_20 or deionized H_20 prior to use. This product is light sensitive.

Note: *To use this buffer with the One Lambda Micro SSP™ Gel System, consult the* ***Directions for Use*** *for the Gel System*

Material Safety Data Sheet

Authorization date: 01/15/2001
Revision date: 09/17/2009
Print Date: 09/17/2009
Version: 1

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product name: AmpliTaq, 75ul (Taq75)
Product number: 04717899001
Business area: Roche Applied Sciences
Product line: None Identified

Supplier:
Roche Diagnostics Corporation
9115 Hague Road
Indianapolis, IN 46250
Site Phone Number: 1-800-428-5074

Emergency telephone number:
CHEMTREC:
1-800-424-9300 (U.S. or Canada)
1-703-527-3887 (International)

2. COMPOSITION/INFORMATION ON INGREDIENTS

Components	CAS Number	Weight %	OSHA PEL:	OSHA STEL:	ACGIH TLV:	ACGIH STEL:
Glycerol	56-81-5	50 - 60	15 mg/m^3 total 5 mg/m^3	NA	10 mg/m^3	NA
EDTA	60-00-4	0.1-<1	NA	NA	NA	NA

3. HAZARDS IDENTIFICATION

Emergency Overview

NFPA Ratings: Health= 1 Flammability= 0 Reactivity= 0 Special= I

Special Definitions: A=Allergen CA=Carcinogen CO=Corrosive F=Flammable H=Harmful I=Irritant Ox=Oxidizer PB=Potential Biohazard R=Reproductive S=Sensitizer T=Toxic T+=Highly Toxic W=Water Reactive

Principle routes of exposure: Ingestion, inhalation, skin and/or eye contact.

Inhalation: May cause irritation of respiratory tract.

Ingestion: Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea.

Skin contact: Substance may cause slight skin irritation.

Eye contact: May cause irritation.

Sensitization or Odor threshold: None.

Medical conditions aggravated by exposure: None known

Additional information: None

4. FIRST AID MEASURES

Inhalation: Consult a physician. Move to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.

Skin contact: Wash off immediately with plenty of water for at least 15 minutes. If skin irritation persists, call a physician.

Ingestion: Call a physician immediately. Do not induce vomiting without medical advice.

Eye contact: Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes.

Notes to physician: None determined

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media: Use dry chemical, CO2, water spray or "alcohol" foam.

Unusual hazards: None known.

Special protective equipment for firefighters: In the event of fire, wear self-contained breathing apparatus.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions: Use personal protective equipment. Evacuate personnel to safe areas. Remove all sources of ignition.

Environmental precautions: Prevent further leakage or spillage if safe to do so. Prevent product from entering drains. Do not flush into surface water or sanitary sewer system. Do not let product enter drains.

Methods for cleaning up: Never return spills in original containers for re-use. Soak up with oil absorbent material. Sweep up or vacuum (if powder) or soak up with inert absorbent material (if liquid), then place into a suitable clean, dry, closed container, and label for disposal.

7. HANDLING AND STORAGE

Handling: Protect from contamination. Wear personal protective equipment.

Storage: No special precautions required.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Engineering measures: Not applicable.

Personal Protective Equipment

Respiratory protection: Respiratory protection is not required under normal use of this product. If respiratory protection is needed, follow the OSHA regulation, 29CFR1910.134. Always use a NIOSH approved respirator when necessary.

Hand protection: Wear appropriate protective gloves to prevent skin contact. Replace torn or punctured gloves promptly.

Skin and body protection: Wear appropriate body protection to prevent skin contact.

Eye protection: Wear appropriate eye protection to prevent eye contact.

Hygiene measures: When using, do not eat, drink or smoke. Keep away from food and drink. Wash hands before breaks and at the end of workday.

9. PHYSICAL AND CHEMICAL PROPERTIES

Boiling point/range: 100C
Melting point/range: 0C
pH: 6-8
Physical state: Liquid.
Flash point: No information available.
LEL (%): Not determined
UEL (%): Not determined

10. STABILITY AND REACTIVITY

Stability: Stable under recommended storage conditions.

Polymerization: Polymerization can occur.

Hazardous decomposition products: Thermal decomposition can lead to release of irritating gases and vapours. Carbon monoxide. Nitrogen oxides (nox).

Materials to avoid: Incompatible with strong acids and bases. Oxidising agents (strong).

11. TOXICOLOGICAL INFORMATION

Components	NIOSH - Selected LD50s and LC50s:	NIOSH Pocket Guide - Target Organs:
Glycerol	=12600 mg/kg Oral LD50 Rat >21900 mg/kg Dermal LD50 Rat >570 mg/m^3 Inhalation LC50 Rat 1h	respiratory system skin eyes kidneys
EDTA	=1700 mg/kg Oral LD50 Rat	

Inhalation: No additional data available

Skin: No additional data available

Oral: No additional data available

Mutagenic effects: No data is available on the product itself
Reproductive toxicity: No data is available on the product itself

12. ECOLOGICAL INFORMATION

Bioaccumulation: Not determined

Aquatic toxicity: No data is available on the product itself

Ecotoxicity effects: No data is available on the product itself

U.S. EPA, RCRA, CERCLA, SARA, or DGC information on persistent, bioaccumulative, and toxic chemicals (PBTs):
The following chemicals are listed under the U.S. EPA regulations of RCRA, CERCLA, SARA, or DGC:

13. DISPOSAL CONSIDERATIONS

Waste from residues / unused products: Waste disposal must be in accordance with appropriate Federal, State, and local regulations. This product, if unaltered by use, may be disposed of by treatment at a permitted facility or as advised by your local hazardous waste regulatory authority. Residue from fires extinguished with this material may be hazardous.

14. TRANSPORT INFORMATION

Is product hazardous to ship? No.

DOT

Proper shipping name: None			
UN Number: Not applicable	**Hazard class:** Not applicable	**Subsidiary risk:** Not applicable	**Packing group:** Not applicable

ICAO/IATA

Proper shipping name: None			
UN Number: Not applicable	**Hazard class:** Not applicable	**Subsidiary risk:** Not applicable	**Packing group:** Not applicable

15. REGULATORY INFORMATION

U.S. Regulations:

U.S. CERCLA/SARA/TSCA Regulatory Information: The following chemicals are listed under the following TSCA/SARA/CERCLA lists. Refer to TSCA regulation if you need a definition for acronyms that may be shown in the TSCA Inventory field in the table below

Components	CERCLA/SARA 302 RQ and TPQ (40 CFR 355, App.A)	CERCLA/SARA 304 RQ (40 CFR Table 302.4)	SARA 313 Emission reporting	TSCA Inventory
Glycerol				Present
EDTA		=2270 kg final RQ		Present

U.S. Clean Water Act (CWA)/ California Proposition 65: The following chemicals are listed under the CWA and/or California Proposition 65:

Components	Clean Water Act Hazardous Substances	Clean Water Act Priority Pollutants	California Proposition 65
EDTA	Present		

Canadian Regulations:

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

Components	Canada - WHMIS: Classifications of Substances:
Glycerol	Uncontrolled product according to WHMIS classification criteria
EDTA	D2B

16. OTHER INFORMATION

Reason for revision:	Not applicable
References:	None.
Additional advice:	None
Prepared by:	Roche Diagnostics, Health & Safety Department, MSDS Contact: 317-521-7425 or 317-521-7505

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End of Safety Data Sheet

Release Specification

xMAP Sheath Fluid
Product Numbers 40-50000 and 40-50015

Sheath Properties

pH	7.30 - 7.50
Conductivity (mS)	16.0 - 18.0
Refractive Index	1.3344 - 1.3354
Bioburden	<20cfu/L per 50mL sample

Manufactured by:
Luminex Corporation
Austin, Texas 78727
www.luminexcorp.com

To order more product
For customer support

fax: 512-219-0544 or email: orders@luminexcorp.com
call: 877-785-2323 (U.S. and Canada) or +1 512-381-4397 (International)
fax: 512-219-5195 or e-mail: support@luminexcorp.com