C1qScreen™

REF **Catalog # C1Q**

IVD For In Vitro Diagnostic Use (European Union and Canada Only)

INTENDED USE

C1qScreen is for the detection of complement-binding HLA antibodies in human serum.

SUMMARY AND EXPLANATION

The C1qScreen kit contains human complement protein C1q, PE-conjugated anti-human C1q, human complement C1q positive control beads, and HEPES buffer.

PRINCIPLE(S)

C1qScreen is used with HLA-coated bead mix to detect complement-binding anti-HLA antibodies in human serum. LABScreen® HLA-coated microbeads and human complement C1q positive control beads are incubated with test serum and human complement C1q. The HLA antibodies bind to the target antigens followed by attachment of C1q to those antibodies that are complement-binding. PE-conjugated anti-human C1q is used as a reporter to indicate the presence of complement-binding antibodies.

The beads are analyzed using either a LABScan™100 (Luminex®100/200) or LABScan3D™ (Luminex FLEXMAP 3D) instrument. The fluorescence intensity (FI) measured on either LABScan instrument indicates the relative amount of antibody bound to the test sample. A significant fluorescent shift indicates a positive reaction. HLA Fusion™ software is available to assist in assigning HLA specificity by comparing the reactivity profile to the bead-antigen panel code.

REAGENTS

A. Identification
- Human Complement C1q: PEPC1Q
- PE Conjugated Anti-C1q: PEPAC1Q
- C1qScreen™ Positive Control Beads: PEPC1QPCB
- HEPES Buffer: HEPBUF

IVD B. Warning or Caution

1. **Warning:** PEPAC1Q contains 0.1% sodium azide as a preservative. Under acidic conditions, sodium azide yields hydrazoic acid, an extremely toxic compound. Dispose in accordance with all federal, state, and local environmental regulations. Refer to the Material Safety Data Sheet (MSDS) for detailed information.
2. **Warning:** All blood products should be treated as potentially infectious. No known test method can offer complete 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.

C. Preparing Reagents for Use

See “Directions for Use,” below

D. Storage Instructions

The entire kit package is shipped on dry ice and should be stored in a freezer at -65°C or below until first use, up to the expiration date.

- Human Complement C1q (Cat. # PEPC1Q): After thawing, store at -20°C up to 2 months (it will not freeze at -20°C).
- PE Conjugated Anti-C1q (Cat. # PEPAC1Q): After thawing, store at 2-8°C up to 2 months. Do not refreeze. Protect from light.
- HEPES Buffer (Cat. # HEPBUF): After thawing, store at 2-8°C up to 2 months. Do not re-freeze.
- C1qScreen™ Positive Control Beads (Cat. # PEPC1QPCB): After thawing, store at 2-8°C for up to 2 months. Do not re-freeze. Protect from light.

E. Purification or Treatment Required for Use

Heat inactivate test serum (see "Directions for Use" below).

F. Instability Indications

None.

INSTRUMENT REQUIREMENTS

A. Required Equipment

- LABScan 100 (Luminex 100/200) instrument with optional Luminex XY platform (for automated 96-sample reading) and sheath fluid delivery system (Cat. # LABSCNXS2) or LABScan 3D (Luminex FLEXMAP 3D) instrument (Cat. # LABSCNXS4).
- Centrifuge
- Swinging centrifuge bucket rotor for 96-well microplate
- Vortex mixer
- Plate shaker or rotating platform

B. Equipment Calibration

- Follow manufacturer's instructions for calibration of the LABScan instrument.[1,2]

C. Recommended Software

- HLA Fusion™ (Cat. # FUSPGR)

SPECIMEN COLLECTION AND PREPARATION

- Separate serum immediately after collection. Store at 2-8°C up to 7 days, at -20°C for up to 11 months. Thaw at 2-8°C overnight and aliquot. Avoid freeze-thaws.
- Use undiluted serum for the test.
- No EDTA or other chemical should be added to the test serum.
- Samples containing contaminants or aggregates may clog the LABScan instrument and generate inaccurate data. Remove aggregates from the test serum by centrifugation or filtering prior to testing.
- Plasma or hemolyzed blood should not be used for the test.

PROCEDURE

A. Materials Provided

- Human Complement C1q (Cat. # PEPC1Q), 25 µL (1 µL/test)
- PE Conjugated Anti-C1q (Cat. # PEPAC1Q), 125 µL (5 µL/test)
- C1qScreen™ Positive Control Beads (Cat. # PEPC1QPCB), 12.5 µL (0.5 µL/test)
- HEPES Buffer, 10X (Cat. # HEPBUF), 100 µL (4 µL/test)

B. Materials Required, But Not Provided

1. LABScreen HLA-coated bead mix
2. Na+, K+-Phosphate-buffered saline (PBS) pH 7.4 (without Ca++ or Mg++)
3. 250 µL 96-well microplate (non-treated surface)

4. Tray seal (OLI Cat. #SSPSEA300 or equivalent)
5. Negative Control Serum, containing no HLA complement binding antibody
6. 1.5mL tubes
7. Optional reagents:
    - C1qScreen Positive Control, Class I (Cat. # C1QS-PC1), 100 µL (5 µL/test)
    - C1qScreen Positive Control, Class II (Cat. # C1QS-PC2), 100 µL (5 µL/test)
    - C1qScreen Negative Control Serum (Cat. # C1QS-NC), 100 µL (5 µL/test)

C. Directions for Use

Notes:

- Turn on the LABScan instrument(s) at least 30 minutes before starting the assay.
- Create a filename and sample code sheet for each 96-well test plate.

1. **Heat Inactivate Test Serum to remove any endogenous C1q**
    a. Add 40 µL of serum to a 1.5 mL tube.
    b. Heat at 56°C for 30 minutes.
    c. Centrifuge at 8,000 – 10,000 g for 10 minutes.
    d. Transfer 30 µL of serum (supernatant) to another 1.5 mL tube. Keep on ice until use.
2. **Prepare Testing Beads**

    Premix the C1qScreen™ Positive Control Beads (PEPC1QPCB) with HLA antigen-coated microbeads. Prepare 10% extra to account for inadvertent pipetting loss.

    a. Calculate the total volume of HLA coated beads needed:

    $Vol_{HLA\ Beads}$ = N (number of tests) X 5 µL bead/test X 1.1 (10% overage)

    b. Calculate the total volume of C1q Positive Control beads needed:

    Vol_{C1QPCB} = N (number of tests) X 0.5 µL bead/test X 1.1 (10% overage)

    c. Add the calculated volume of C1q Positive Control Bead to the volume of HLA coated bead in a 1.5 mL tube.
    d. Mix solution by vortexing. Keep on ice until use.
3. **Prepare the C1q**

    Make 5-fold dilution of Human Complement C1q (PEPC1Q) in HEPES buffer (HEPBUF) prior to the test. Prepare 10% extra to account for inadvertent pipetting loss.

    a. Calculate the total volume of C1q needed:

    Vol_{C1q} = N (number of tests) X 1 µL C1q/test. X 1.1 (10% overage)

    b. Calculate the volume of HEPES buffer needed:

    Vol_{HEPBUF} = N (number of tests) X 4 µL HEPBUF/test. X 1.1 (10% overage)

    c. Add the C1q to the HEPES buffer in a 1.5 mL tube.
    d. Mix solution by vortexing. Keep on ice until use.
4. **Test Procedure**

    Note*: Use known positive and negative reference sera to determine assay cut-off. C1qScreen Positive Controls for HLA Class I or Class II tests (C1QS-PC1 or C1QS-PC2), can be used as control samples to monitor kit reactivity. Use 5 µL per test (heat inactivation is not required).*

    a. Vortex the tubes of C1q, testing beads, and test sera to ensure proper mixture of the reagents.
    b. Add 5 µL of heat-inactivated test serum to designated wells of a 96-well plate.

c. Add 5 µL of diluted Human Complement C1q (PEPC1Q) to each well of a 96-well plate.

d. Add 5 µL of the premixed beads to each well of a 96-well plate.

e. Cover with a tray seal and vortex to mix.

f. Incubate for 20 minutes at room temperature on a shaker. Cover to protect from light.

g. After the incubation, pulse spin the 96-well plate in a centrifuge up to 1000 g to ensure no liquid remains on the tray seal.

h. Vortex PE Conjugated Anti-C1q (PEPAC1Q) to mix.

i. Remove tray seal and add 5 µL of PE Conjugated Anti-C1q (PEPAC1Q) to each test well.

j. Cover with tray seal and vortex to mix.

k. Incubate for 20 minutes at room temperature on a shaker. Cover to protect from light.

l. Pulse spin the tray in a centrifuge up to 1000 g to ensure no liquid remains on the tray seal.

m. Remove tray seal and add 80 µL of PBS to each test well.

n. Centrifuge at 1,300 g for 5 minutes to pellet the beads.

o. Remove supernatant by flicking the 96-well plate.

p. Add 80 µL of PBS to each testing well. Cover with tray seal and vortex to mix.

q. Pulse spin the tray in a centrifuge up to 1000 g to ensure no liquid remains on the tray seal.

r. Remove tray seal and read sample fluorescent intensity with LABScan 100 or LABScan 3D instrument, per Data Acquisition section below.

RESULTS

A. Data Acquisition

1. Set up the LABScan instrument for sample acquisition and calibration according to the Luminex User's Manual.[1,2]
2. Choose a template/protocol according to product kit catalog and lot numbers.
3. Acquisition templates/protocols are available from One Lambda, Inc. at our download website. To create your own acquisition template, refer to the Acquisition chapter of the Luminex Users Manual.[1,2]
4. Create a file name for the samples to be run.
5. Make sure all the template settings are correct. 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 lot-specific worksheet provided with the HLA-coated bead product.
    e. Set minimum events collected to 100 per bead.
6. Enter the sample IDs. (**Caution:** If the same sample is tested more than once, assign different IDs.)
7. Load the plate onto the XY platform and fill the reservoir with sheath fluid.
8. Click the START button to initiate the session. After the samples have finished running, the data output is saved as a .csv file.
9. Wash the machine twice with sheath fluid at the end of the session.

B. Data Analysis

1. Serum reactivity can be assessed by the fluorescent signals from the HLA coated beads, after correction for non-specific binding to the negative control (NC) bead (#001).
2. Results are normalized using a negative control serum (e.g., OLI Cat. # C1QS-NC).

3. The reactivity of a test sample is calculated from the trimmed mean fluorescence values, which are reported in the LABScan .csv file output.
4. The background values for each bead may be obtained by testing with a negative control serum.

C. Calculations

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

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

2. Reactivity can be calculated with HLA Fusion software or by the following formulas:

Baseline = (S#N – SNC bead) – (BG#N - BGNC bead)

LIMITATIONS OF THE PROCEDURE

- Serum samples only. Plasma samples with added anti-coagulants may give different MFI values from serum. For example, ACD and EDTA decreased weak positive MFI signals and increased background. Heparin (Li or Na) significantly increased MFI.
- The following substances have been shown to affect the MFI signal: nicotine, ibuprofen, cholesterol, creatinine, and hemoglobin. The effects of these substances were modest, except for hemoglobin (e.g. hemolyzed blood), which significantly increased the MFI signal.
- Accurate data acquisition depends on proper performance of the LABScan instrument. The machine must be properly calibrated and maintained. Insufficient flushing of the system may allow aggregates in the sample to clog the machine and generate invalid data. If the ambient temperature changes, the machine may need re-calibration. Consult the Luminex manual[1, 2].
- Assignment of antibody specificity is limited to the HLA antigens presented in the specific LABScreen product 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 lot-specific worksheet.)
- Due to the complexity of HLA, only certified HLA technicians/specialists should review and interpret data. Perform HLA testing in accordance with ASHI and EFI regulations.
- This test should not be used as the sole basis for clinical decisions.

EXPECTED VALUES

- Each bead count should be over 50. A lower bead count may be due to sample loss during the wash step, improper calibration, pipetting error, clogging of the LABScan instrument, or photo-bleached beads that dropped out from the mapped region.
- Signal values are the trimmed mean fluorescence intensity of reactions between the bead set and the test serum. A negative control serum should be tested with the same batch of samples to establish the background value(s) for the test run.
- Using any negative control serum other than OLI negative control serum (Cat. # C1QS-NC) 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 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 bead value.
- C1q Positive Control Beads (Ag ID = PC) are beads coated with C1q, which should bind to the secondary antibody to produce a positive signal. The PC trimmed mean fluorescent value must be over 500 and at least twice the NC value. Only the trimmed mean fluorescent value for PC beads should be evaluated, because the median fluorescent value for PC beads may be lower than 500 when C1q positive control beads are mixed with LABScreen PC beads. However, the trimmed mean fluorescent value for PC beads should be over 500.

- For a given serum, the mean 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 Negative Control serum, a low signal from the secondary antibody, or the LABScan system. In this case, the data may have to be confirmed.
- 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.
- To determine the specificity of HLA antibody, enter the reaction score into the lot-specific worksheet to analyze the reaction pattern; or use HLA Fusion software.
- Plasma samples may give lower MFI or higher background values than serum, and can generate inaccurate results. We recommended not using plasma samples, as indicated in Specimen Collection and Preparation.

SPECIFIC PERFORMANCE CHARACTERISTICS

- C1qScreen has demonstrated similar or better sensitivity than Lambda Cell Tray™ (LCT™) assays based on internal studies, using comparisons of 152 samples between C1qScreen and LCT™.
- Most of the reactivity detected by C1qScreen was detected by LABScreen, based on internal studies testing 152 samples with both C1qScreen and LABScreen assays. LABScreen detects both complement binding and non-complement binding IgG antibodies. However, IgM (which is also effective at binding complement) is not detected by LABScreen (which uses anti-IgG-PE as the 2nd antibody).
- 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 assay cut-offs for its own use, based on its expertise in recognizing HLA CREG patterns and an evaluation of the assay performance, using HLA allosera with defined specificities.

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TRADEMARKS AND DISCLAIMERS

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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
CE 0197	CE mark of medical quality
	Manufacturer
EC REP	Authorized representative in the European Community

REVISION HISTORY

Revision	Date	Revision Description
7	05/09/2017	Removed instruction to protect from light under Page 2 Specimen Collection and Preparation.
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.

CE 0197