Anti-HLA Class I Control Kit

Ready-to-use kit: positive control antibody for class I Luminex® (LABScreen® or LIFECODES HLA)
Catalog # kc-hla-c1
For research use only
Version # 16E24-MM

PRODUCT INFORMATION

Content
Sterile Anti-HLA Class I Control antibody provided lyophilized

Each vial contains enough anti-HLA for 25 tests.
- 3 vials of Anti-HLA Class I Low for “low MFI”
- 3 vials of Anti-HLA Class I Medium for “medium MFI”
- 3 vials of Anti-HLA Class I High for “high MFI”
- 1 vial of sterile water

Mean Fluorescence Intensity (MFI)

Formulation
Anti-HLA Class I Control antibody was lyophilized from a 0.2 μm filtered phosphate buffer solution (pH 7.4) containing human serum albumin (HSA) and 0.01% w/v sodium azide.

Storage and stability
- The kit is shipped at room temperature and must be stored at -20°C upon receipt.
- Lyophilized products are stable for 12 months at -20°C.
- Upon resuspension, store at 4°C. Resuspended product is stable for 3 months at 4°C when properly stored. Do not freeze the resuspended product. Note: The vial must be free of contamination. Discard the vial if any turbidity, color or sediment appears.

QUALITY CONTROL

This monoclonal antibody is produced by recombinant technology in CHO cells and prepared under strict aseptic conditions. The Anti-HLA Class I Control kit is validated in LABScreen® Mixed (Figure 1; on the next page) and LABScreen® Single Antigen HLA Class I assays (Figure 2; on the next page).

APPLICATIONS

The Anti-HLA Class I and the Anti-HLA Class II (cat. code #kc-hla-c2) Control Kits contain positive control antibodies to be used for the validation and standardization of anti-HLA multiplex bead array assays using Luminex® technology (LABScreen®, LIFECODES HLA, C1qScreen®). These kits contain 3 different concentrations of chimeric human monoclonal IgG1 (US patent application 2013/0288387) that react with the antigen beads to generate non-saturating low, medium and high MFI.

DESCRIPTION

The Anti-HLA Class I Control kit comprises ready-to-use dilutions of monoclonal antibody for use as positive control in solid-phase antigen bead assays for detection of anti-HLA human antibodies (LABScreen® and LIFECODES HLA).

Detection of anti-HLA antibodies by Luminex® has become a reference technique for minimizing the risk of humoral acute rejection in allografts. This semi-quantitative technique enables monitoring of patients by tracking the MFI of specific antigenic beads. In order to standardize this assay and to enable interpretation of low MFI, each lab will typically use as positive control, a mixture obtained by pooling the sera from multiple poly-immunized patients. However, such in-house control samples are limited in quantity, do not give a homogenous signal across all HLAs and show batch-to-batch variability. Therefore, there has been a longstanding need for a universal control that would be available in unlimited quantity and that could enable standardization of results and methods across laboratories.

InvivoGen, together with Professor Antoine Blancher (Histocompatibility and Immunogenetics Laboratory, Toulouse University Hospital), has developed universal positive-control kits for the calibration of Luminex® assays. They enable accurate interpretation of MFI values and validation of cross-matching experiments. The positive controls are biotechnologically produced human chimeric IgG1 antibodies that recognize either class I or class II human HLA antigens. The anti-HLA class I IgG1 variable regions is derived from the murine monoclonal antibody W6/32. Similarly to patient antibody, this monoclonal is labeled with the same secondary anti-human IgG used for the anti-HLA antibody detection (PE-conjugated anti-human IgG).

The Anti-HLA Class I Control kit contains the Anti-HLA Class I Control antibody at three different concentrations for standardization of low, medium and high MFI values.

The Anti-HLA Class I Control kit has been validated in LABScreen® Mixed and LABScreen® Single Antigen HLA Class I assays. It reacts with all class I antigens (HLA-A, B and Cw antigens). The anti-HLA Class I control also binds strongly to recombinant C1q using the C1qScreen® kit.


TECHNICAL SUPPORT
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METHODS
Preparation of resuspended antibody solutions
- Add 500 μl of sterile water at room temperature.
- Gently invert the vial. Avoid vigorous shaking and foam formation.
- Store at 4°C (stable for 3 months).

Use as positive control for anti-HLA Luminex® assay (LABScreen® and LIFECODES HLA)
To standardize your results, we recommend the use of a low, medium and high MFI positive control per run. Typical MFI results obtained with the anti-HLA class I control kit for high (A), medium (B) and low (C) MFI positive control vials using LABScreen® Mixed can be seen on Figure 1 and LABScreen® Single Antigen HLA Class I on Figure 2.

- Add 20 μl of anti-HLA antibody to 5 μl of beads following manufacturer’s instruction.

The tests on LABScreen® are performed according to the manufacturer’s instructions. Briefly, MultiScreen Filter Plate is first moistened with 300 μl of wash buffer and then incubated for 10 min. After resuspension, 5 μl of LABScreen® beads is added to each well followed by the addition of 20 μl of anti-HLA. The mixtures beads-anti-HLA antibody solutions are then incubated for 30 min and then washed 5 times. A 100-μl aliquot of conjugate anti-human IgG-PE conjugated (diluted at 1:100) is then added to each well. The resulting mixtures are incubated for 30 min at room temperature and protected from light. After 5 washes, 80 μl of PBS is added to each well. Finally, MFI values for each bead are acquired using LabScan software.

Figure 1: Typical fluorescent response obtained with the anti-HLA class I control kit for high (A), medium (B) and low (C) MFI positive on LABScreen® Mixed labeled with a PE-conjugated anti-human IgG. The data are average MFI values for the class I beads. The class II beads signal (not shown) was at background level.

Figure 2: Printouts from HLA Fusion software showing single antigen bead histogram of HLA class I. Binding specificity and intensity was assessed for the anti-HLA class I control kit for low (A), medium (B) and high (C) MFI positive control vials using LABScreen® Single Antigen HLA Class I (LABScan 100). As can be seen all class I antigens are recognized by the anti-HLA class I.