Anti-HLA Class I Control

Human monoclonal IgG1 positive control for anti-HLA class I Luminex® (LABScreen® or LIFECODES HLA)

Catalog code: hla-c1

https://www.invivogen.com/anti-hla-class1

For research use only

Version 19I16-MM

PRODUCT INFORMATION

Content

- 100 µg sterile Anti-HLA Class I Control antibody, provided azide-free and lyophilized

Note: The antibody dilution buffer is not provided.

Storage and stability

- Anti-HLA Class I Control is provided lyophilized and shipped at room temperature. Store the antibody at -20 °C.
- Lyophilized product is stable for 24 months at -20 °C when properly stored.
- Upon resuspension, store Anti-HLA Class I Control 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 is validated in LABScreen® Mixed and LABScreen® Single Antigen HLA Class I assays. Typical Mean Fluorescence Intensity (MFI) values obtained with antigen beads are shown in figures 1 and 2 (on the next page).

DESCRIPTION

Anti-HLA Class I Control is a chimeric monoclonal antibody. Its constant regions are from human IgG1 (gamma 1 heavy chains and kappa light chains) and its variable regions are derived from the murine monoclonal antibody W6/32[1].

Anti-HLA Class I Control reacts with all HLA class I antigens tested[2,3] (class I HLA-A, B and Cw antigens tested on LABScreen® Single Antigens).

1. Barnstable CJ. *et al.*, 1978. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens-new tools for genetic analysis. Cell. 14(1):9-20. **2. Hilton HG. & Parham P., 2013.** Direct binding to antigen-coated beads refines the specificity and cross-reactivity of four monoclonal antibodies that recognize polymorphic epitopes of HLA class I molecules. Tissue Antigens. 81(4):212-20. **3. Congy-Jolivet N. *et al.*, 2013.** Production and characterization of chimeric anti-HLA monoclonal antibodies targeting public epitopes as tools for standardizations of the anti-HLA antibody detection. J Immunol Methods. 390(1-2):41-51.

APPLICATIONS

Anti-HLA Class I Control and Anti-HLA Class II Control (cat. code hla-c2) were specifically designed for the validation and standardization of the detection of antibodies to HLA class I and class II (US patent application 2013/0288387). Both antibodies can be used as positive controls for all anti-HLA antibody detection assays, including:

- multiplex bead array assays using the Luminex® technology (LABScreen® and LIFECODES HLA);
- ELISA;
- indirect immunofluorescence techniques (flow cytometry);
- complement-dependent lymphocytotoxicity; and
- C1q Screen™.

METHODS

Preparation of stock solution (100 µg/ml)

- Add 1 ml of sterile distilled water at room temperature.
- Gently invert the vial. Avoid vigorous shaking and foam formation.
- Prepare aliquots and store at 4 °C.
- Prepare dilutions using the antibody dilution buffer (see below) for immediate use.

Note: Do not store dilutions. Discard after use.

Use as a control for Luminex® assay (LABScreen® & LIFECODES HLA)

Anti-HLA Class I Control and Anti-HLA Class II Control have been validated as positive controls for the detection of antibodies to HLA class I and class II, respectively, using LABScreen® Mixed and LABScreen® Single Antigen.

Anti-HLA Class I Control and Anti-HLA Class II Control stock solutions can be mixed without risk of interference for validating and standardizing the detection of anti-HLA antibodies.

Titration curves are typically obtained based on serial dilution in phosphate buffered saline (PBS) supplemented with 6% albumin (figures 1 & 2 on the next page).

This anti-HLA Class I Control antibody can also be used as a positive control in C1q Screen™ (figure 3 on the next page).

Antibody dilution buffer: Phosphate buffered saline without Ca^{2+} supplemented with 6% albumin (highly purified human serum albumin or ultrapure bovine serum albumin).

Working concentration: 0.2 ng/ml – 2 µg/ml

20 µl of different dilutions of the antibody solution should be used. As can be seen in the titration curve (figure 1), typical final IgG concentrations of 500 ng/ml, 50 ng/ml and 5 ng/ml will result in low, medium and high non-saturating MFI.

TECHNICAL SUPPORT
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Luminex® Protocol:

The tests on LABScreen® are performed according to the manufacturer's instructions. Briefly, a MultiScreen Filter Plate is first moistened with 300 µl wash buffer, and then incubated for 10 min. After resuspension, 5 µl of LABScreen® beads are added to each well, followed by 20 µl of anti-HLA class I or class II IgG. The resulting mixtures are incubated for 30 min, and then washed 5 times. A 100-µl aliquot of anti-human IgG-PE (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 are added to each well. Finally, mean fluorescence intensity (MFI) values for each bead are acquired using LabScan software.

Use as a control for other immunoassays

We recommend to use the antibody dilution buffer and the working concentration as described on the previous page. Detailed protocols have been published (Congy-Jolivet *et al.*, 2013. Ref. on the previous page).

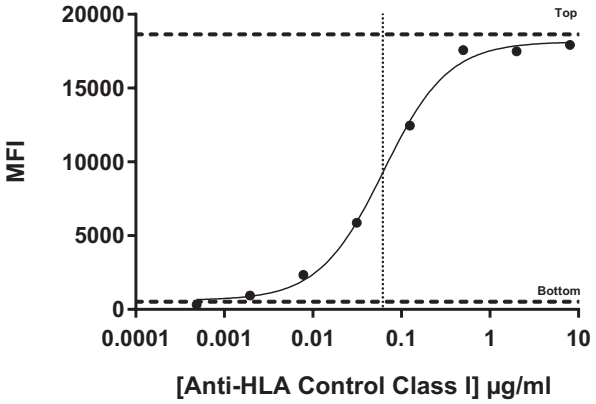

Figure 1: Typical fluorescent dose response obtained by serial dilution of Anti-HLA Class I Control (hIgG1) antibody (µg/ml) on LABScreen® Mixed labeled with a PE-conjugated anti-human IgG. The curve is a plot of average MFI values for the class I beads (minimal standard deviation as each bead gave a very similar value). The class II beads signal (not shown) was at background level.

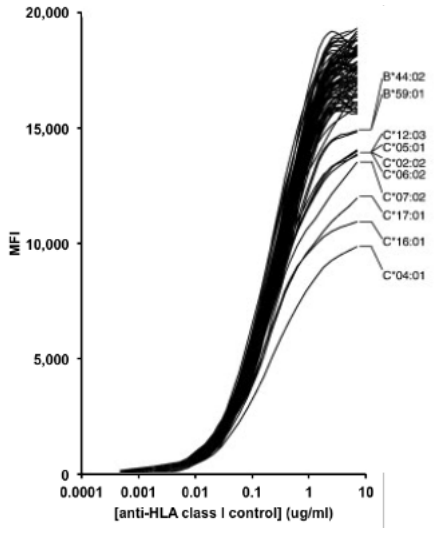

Figure 2: Fluorescent (MFI) dose response obtained by binding of anti-HLA Class I Control (hIgG1) to LABScreen® Single Antigen beads. Each curve is representative of a specific HLA antigenic bead. Antigens that gave the lowest values are indicated on the right. As observed in the graph all class I antigens are recognized by the anti-HLA Class I Control.

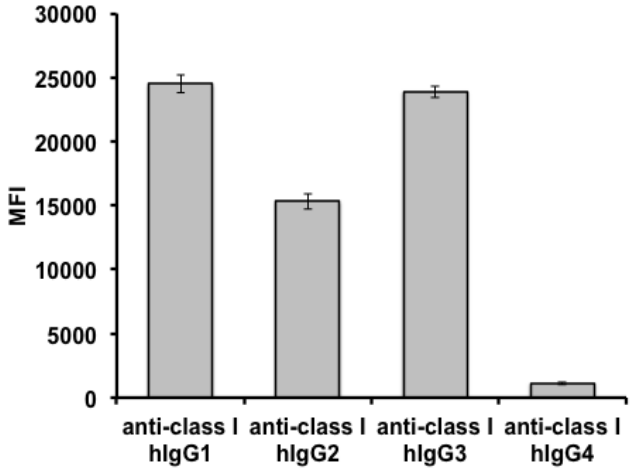

Figure 3: Average fluorescent intensity obtained at 1 µg/ml of Anti-HLA Class I Control (hIgG1) on LABScreen® Mixed and C1qScreen™. The data are average MFIs of the class I beads. Human IgG2 (hIgG2), hIgG3 and hIgG4 anti-HLA antibodies were produced separately to assess sensitivity. The class II beads signal (not shown) was at background level.