Human IgE ELISpot B Cell Development Module

Catalog Number: SELB001

Reagents Provided

Human IgE Capture Antibody Concentrate (Part # 843214): 1 vial of lyophilized goat anti-human IgE polyclonal antibody.*

Human IgE Detection Antibody Concentrate (Part # 843215): 1 vial of lyophilized biotinylated goat anti-human IgE polyclonal antibody.*

*Each vial contains sufficient antibodies to run ELISpot assays on approximately five 96-well microplates, when using the protocol provided.

Reagent Preparation and Storage

Capture Antibody Concentrate: Reconstitute with 1 mL of PBS. After reconstitution, store at 2-8 °C for up to 60 days or aliquot and store at -20 °C to -70 °C in a manual defrost freezer for up to 6 months.

Detection Antibody Concentrate: Reconstitute with 1 mL of Reagent Diluent. After reconstitution, store at 2-8 °C for up to 60 days or aliquot and store at -20 °C to -70 °C in a manual defrost freezer for up to 6 months.

For optimal performance, prepare the working dilutions of the Capture and Detection Antibodies immediately before use.

Other Supplies Required

- ELISpot Blue Color Module (R&D Systems, Catalog # SEL002) or equivalent
- Antigen (or Biotinylated Antigen) of interest (if biotinylated antigen is not available, ChromaLink™ Biotin Labeling Kit (SoluLink™, Catalog # B-9007-105K) or equivalent is also recommended)
- PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2-7.4, 0.2 μm filtered)
- Wash Buffer (0.05% Tween® 20 in PBS)
- Blocking Buffer (1% BSA, 5% Sucrose in PBS)
- Reagent Diluent (1% BSA in PBS, pH 7.2-7.4, 0.2 μm filtered)
- 2-8 °C refrigerator
- 37 °C CO_2 incubator
- Positive Control - For use with the total IgE protocol (below)
- 96-well microplates (PVDF-bottom MultiScreen® microplates; Millipore, Catalog # MSIPS4W10 or equivalent)
- Squirt bottle, manifold dispenser, or automated microplate washer
- Dissection microscope or an automated ELISpot reader
- Deionized H_2O

ELISpot Protocol

Three different protocols may be used with this kit to study IgE secretion from stimulated memory B cells. See references 1-3.

A) Plates are coated with the antigen that will capture secreted IgE and the provided biotinylated anti-IgE antibody is used as the detection reagent. The investigator supplies the antigen of interest. This protocol allows the user to determine the frequency of B cells secreting antigen-specific IgEs.

B) Plates are coated with the provided anti-IgE antibody that will capture secreted IgE from B cells, and a biotinylated antigen is then used as a detection reagent. The investigator supplies the biotin conjugated antigen of interest. This protocol allows the user to determine the frequency of B cells secreting antigen-specific IgEs.

C) Plates are coated with the provided anti-IgE antibody that will capture secreted IgE from B cells, and the provided biotinylated anti-IgE antibody is then used as a detection reagent. This protocol allows the user to determine the total amount of secreted IgE.

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When a 96-well PVDF microplate is used, 1:60 dilutions of the provided capture and detection antibodies are recommended. **Each investigator should determine the optimal working dilution of the antibodies depending on the type of microplate, Wash Buffer, and Blocking Buffer used.**

1. **A.** Calculate the total volume of antigen needed to coat the microplate (e.g. 1-50 µg/mL in PBS).
   B. or C. Calculate the total volume of Capture Antibody needed and dilute to the working concentration in PBS.
2. Immediately add 100 µL of the diluted Capture Antibody or antigen per well. Cover the microplate with the lid and incubate overnight at 2-8 °C.
3. Aspirate each well and wash 3 times with Wash Buffer or PBS (350 µL/well) using a squirt bottle, manifold dispenser, or autowasher. After the final wash, remove any remaining liquid by inverting the microplate and blotting it against a clean paper towel. **Note:** *To avoid damage, do not touch the membranes during washing.*
4. Block membranes by adding 200 µL of Blocking Buffer to each well. Incubate for 2 hours at room temperature.
5. Aspirate or decant Blocking Buffer. Rinse with the same media in which the cells will be cultured. **Note:** *Do not discard the culture media until cells are ready to be plated.*
6. Aspirate culture media from the microplate, and immediately fill the appropriate wells with 100 µL of culture media containing human IgE secreting cells. Incubate at 37 °C in a 5% CO_2 incubator. The incubation time must be determined empirically. **Note:** *It is recommended that a positive control (total IgE), negative control (same number of unstimulated cells as stimulated cells), and background control (sterile culture media) be run with each assay.*
7. Wash the microplate 4 times with Wash Buffer. Remove any remaining Wash Buffer by inverting the microplate and blotting it against a clean paper towel.
8. **A. or C.** Calculate the total volume of Detection Antibody needed and dilute to the working concentration in Reagent Diluent.
   B. Calculate the total volume of biotinylated antigen needed (e.g. 0.01-1 µg/mL) in Reagent Diluent.
9. Add 100 µL of the diluted Detection Antibody or biotinylated antigen per well. Cover the microplate with the lid and incubate overnight at 2-8 °C.
10. Aspirate Detection Antibody and wash as described in step 3. Microplates are now ready for color development.

Color Development

Color development may be done using the ELISpot Blue Color Module (see the Other Supplies Required section). Alternatively, another chromogen of choice may be used.

References

1. Lipsky, P.E. (1990) Res. Immunol. **141**(4-5):424.
2. Venkataraman, C. *et al*. (1999) Immunol. Lett. **69**(2):233.
3. Glaum, M.C. *et al*. (2009) J. Allergy Clin. Immunol. **123**(1):224.