



## Mouse IgG B Cell ELISpot Development Module

Catalog Number: SELB004

### Reagents Provided

**Mouse IgG Capture Antibody Concentrate** (Part # 843840):  
1 vial of lyophilized goat anti-mouse IgG polyclonal antibody.\*

**Mouse IgG Detection Antibody Concentrate** (Part # 843841):  
1 vial of lyophilized biotinylated goat anti-mouse IgG 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<sub>2</sub>HPO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, 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<sub>2</sub> incubator
- Positive Control - For use with the total IgG 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<sub>2</sub>O

### ELISpot Protocol

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

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

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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  $\mu\text{g}/\text{mL}$  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  $\mu\text{L}$  of the diluted Capture Antibody or antigen per well. Cover the microplate with the lid and incubate overnight at 2-8  $^{\circ}\text{C}$ .
3. Aspirate each well and wash 3 times with Wash Buffer or PBS (350  $\mu\text{L}/\text{well}$ ) 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  $\mu\text{L}$  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  $\mu\text{L}$  of culture media containing mouse IgG secreting cells. Incubate at 37  $^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  incubator. The incubation time must be determined empirically. **Note:** *It is recommended that a positive control (total IgG), 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  $\mu\text{g}/\text{mL}$ ) in Reagent Diluent.
9. Add 100  $\mu\text{L}$  of the diluted Detection Antibody or biotinylated antigen per well. Cover the microplate with the lid and incubate overnight at 2-8  $^{\circ}\text{C}$ .
10. Aspirate Detection Antibody or biotinylated antigen 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.

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