User Manual

Hybridoma Subisotyping Kit, Mouse

386445

Upon arrival store the entire contents of the kit at 4°C.

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Product Overview

The Hybridoma Subisotyping Kit is intended for the detection and identification of mouse monoclonal antibody subclasses IgG, IgG, IgG, IgM, and IgA in supernatants. It is also used for confirmation of monoclonality of cloned hybridoma cultures. This double antibody detection system is a highly sensitive enzyme immunoassay method incorporating specific primary antisera. Assay sensitivity is less than 1 µg/mL and can be reliably used with diluted culture supernatants. The following protocol utilizes 50 µL X 7 (350 µL) of each supernatant tested (a 1:2 dilution occurs as each sample is added to diluent in each well). When supernatant medium is precious, the medium from high producer cells can be diluted much more (1:10 or greater), with appropriate modifications in the Application of Supernatants section. When used as directed, the reagents in this kit will be sufficient for 900 determinations or 150 clones.

Materials Provided

- Goat Anti-Mouse IgG (KP4001): (Plate Coating Reagent) 3 mL
- Rabbit Anti-Mouse IgG_1 (KP4002): 15 mL
- Rabbit Anti-Mouse IgG_{2a} (KP4003): 15 mL
- Rabbit Anti-Mouse IgG_{2b} (KP4004): 15 mL
- Rabbit Anti-Mouse IgG_3 (KP4005): 15 mL
- Rabbit Anti-Mouse IgM (KP4007): 15 mL
- Rabbit Anti-Mouse IgA (KP4006): 15 mL
- Goat Anti-Rabbit IgG, Peroxidase Conjugated (KP4008): 250 µL
- Plate Coating Solution (KP4009): 15 mL
- Blocking Serum (KP4010): 50 mL
- TMB Substrate (KP4011): 125 mL
- PBS Concentrated Buffer Solution (KP4012): 2 X 125 mL
- Surfactant (KP4013): 1.25 mL

Protocol

Application of Capture Antibody

1. Dilute Stock Plate Coating Solution with distilled water, using 9.0 mL distilled water with 1.0 mL coating solution for each plate to be coated.
2. Add 100 µL of Goat Anti-Mouse IgG (Plate Coating Reagent) to the 10 mL of Plate Coating Solution.
3. Add 100 µL of this plate coating mixture to each well of a 96-well EIA plate.
4. Stack or seal plates and incubate 18-24 hours at 4 °C. If you will not use plates immediately, shake out contents and allow to air dry; cover with sealing film. Plates prepared in this manner have been shown to remain usable for several weeks.

Application of Supernatants

1. Dilute PBS concentrate using 5 mL concentrate for each 100 mL of buffer required. For every liter of buffer, add 500 µL of surfactant to reduce non-specific binding. (This buffer will be used in steps 4 and 6 and in step 1 of Application of Conjugate) A 500 mL squeeze bottle is a convenient container for the PBS-surfactant for the washing steps.
2. Remove coated plate(s) from refrigeration, shake out the contents into a sink and pat dry on a clean towel.
3. Wash the plate(s) with PBS-surfactant using a gentle stream from the squeeze bottle and fill each well. Be sure that no air bubbles are trapped in any of the wells that could prevent complete washing and may result in an incorrect reading at the end of the test. Pat dry with a clean towel.
4. Wash twice more (repeating step 3).
5. Dilute blocking serum 1:4 with 1X PBS.
6. Add 200 µL of diluted blocking serum to each well.
7. Incubate plate(s) at room temperature for 1 hour.
8. Wash the plate(s) with PBS-surfactant and pat dry with a clean towel.
9. Add 50 µL of each hybridoma supernatant to one column of 8 wells. (We suggest using mouse serum diluted 1:500 as a positive control in one column.)
10. Incubate plate(s) at room temperature for 1 hour.

Application of Subisotyping Antisera

1. Shake out contents of the incubated plate(s) and pat dry.
2. Wash plate(s) with water, saline, or PBS and pat dry. Again, check that no air bubbles are trapped.
3. Wash twice more.
4. Add two drops from each of the typing antisera bottles to a different well for each hybridoma tested (e.g. anti-IgM to each well of the top row across, anti-IgA to each well of the second row, etc.).
5. Add 100 µL of PBS-surfactant to any wells that do not receive antiserum. These wells will be negative controls.
6. Incubate plate(s) at room temperature for 1 hour.

Application of Conjugate

7. Dilute the peroxidase conjugate 1:4,000 with PBS-surfactant (add 2.5 µL of conjugate to 10 mL of PBS-surfactant).
8. Shake out the contents of the antisera incubated plate(s) and pat dry.
9. Wash plate(s) with saline or PBS and pat dry. Repeat washes twice more.
10. Add 100 µL of diluted conjugate to each well.
11. Incubate plate(s) at room temperature for 1 hour.
12. Shake out contents of plate(s) and pat dry.
13. Wash plate(s) with saline or PBS and pat dry. Repeat washes twice more.

Application of Substrate

The ready-to-use TMB substrate reagent is stable at 4 °C and produces a blue color that can be read at 655 nm. Addition of acid as a stop solution enhances sensitivity 2-4 times and produces a yellow color that can be read at 450 nm. TMB is light sensitive and should be stored in the dark. For best results, remove the amount needed and transfer to a clean container before adding to plates. Always use a clean pipette tip to remove reagent from bottle to avoid contamination. The solution is clear to very faint blue (contamination turns the solution blue).

1. Using the substrate reagent as supplied, add 100 µL to each well.
2. Color development should be rapid and may be complete in 5-10 min.
3. Positive wells will develop a bright blue color; negative wells will retain a clear to faint blue color. Read plate at 655 nm.
4. Stop color development (or enhance sensitivity) by adding 50 µL of 1 M phosphoric acid to each well and read at 450 nm.

Plate Configuration

	Culture Supernatants											
	1	2	3	4	5	6	7	8	9	10	11	Mouse serum
Goat Anti-Mouse IgG												
Rabbit Anti-Mouse IgM												
Rabbit Anti-Mouse IgG1												
Rabbit Anti-Mouse IgG2a												
Rabbit Anti-Mouse IgG2b												
Rabbit Anti-Mouse IgG3												
Rabbit Anti-Mouse Anti-IgA												
Goat Anti-rabbit IgG												

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