MULTI SCIENCES

Mouse Ig Isotyping ELISA Kit

【Catalog No】EK279

【SIZE】48T/96T

【INTENDED USE】For the qualititative determination of mouse Ig isotypes in hybridoma supernates, ascites and purified antibodies.

【INTRODUCTION】

Antibody isotyping is a critical and beneficial aspect of hybridoma development. This kit can identify six immunoglobulin heavy chain isotypes and two light chain isotypes in mice: IgA, IgM, IgG1, IgG2a, IgG2b, IgG3, kappa chain and lambda chain. It can accurately and specifically identify which heavy and light chain of the hybridoma samples is producing and if it is monoclonal or not. This kit is a powerful tool to isolate and characterize each potential clone.

Identification is essential since chemical and biological properties of the various classes are unique. They differ in their solubility and electrophoretic properties, susceptibility to cleavage enzymes, and reactivity with protein A. Determining the class and subclass of a monoclonal antibody is thus useful in planning the best immunoglobulin purification method. For example, mouse IgA and IgM are best purified by size (i.e., gel exclusion) or using immunoaffinity separation columns. Mouse IgG2a and IgG2b are purified with immobilized Protein A at pH 7 - 8, while mouse IgG1 binds best to Protein A at pH 8 - 9. Immunoglobulin that contains kappa light chains can be purified using immobilized Protein L.

【PRINCIPLE OF THE ASSAY】

This assay employs the qualitative sandwich enzyme immunoassay technique. Monoclonal antibodies specific for mouse Ig isotypes have been pre-coated onto a microplate. Samples, positive control and HRP-linked detect antibody are pipetted into the wells and Ig present is bound by the immobilized antibody and detect antibody following incubation. After washing away any unbound substances, substrate solution is added to the wells and color development is performed. Finally it is stopped and the intensity of the color is measured.

【MATERIALS PROVIDED】

PART	PART #	EK279-48	EK279-96
Coated Microplate	EK279P	48T	96T
Detect antibody	EK279D	1 vial	1 vial
Positive Control	EK279PC	1 vial	2 vials
Assay Buffer (10×)	E0310	5ml	5ml
TMB	E0230	6ml	11ml
Stop Solution	E0300	11ml	11ml
Washing Buffer (20×)	E0281	50ml	50ml
Adhesive Film	E0200	6	6

Note: Components from reagent kits of different batch numbers must not be used interchangeably. Positive Control include mouse IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, kappa and lambda isotype control mix.

OTHER SUPPLIES REQUIRED

1) Microplate reader capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.

2) Pipettes and pipette tips.

3) 50 µl to 300 µl adjustable multichannel micropipette with disposable tips.

4) Multichannel micropipette reservoir.

5) Beakers, flasks, cylinders necessary for preparation of reagents.

6) Deionized or distilled water.

7) Polypropylene test tubes for dilution.

【STORAGE】

Store at 2-8℃; refer to the kit label for expiration date.

For opened kits:

Pre-coated microplate: Can be stored at 2-8℃ for approximately 1 month (return unused strips to the aluminum foil bag and reseal).

Standard: Can be stored at -20℃ for approximately 1 month (discard after single-use reconstitution).

Other components: Can be stored at 2-8℃ for approximately 1 month.

【SAMPLE COLLECTION AND STORAGE】

1) **Cell Culture Supernates** – Remove particulates by centrifugation at 300 × g for 10 minutes and assay immediately or aliquot and store samples at ≤ -20℃.

2) **Other biological samples** might be suitable for use in the assay. Cell culture supernates, acites and purified antibodies were tested with this assay.

Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20℃ to avoid loss of bioactive mouse Ig (H+L). If samples are to be run within 24 hours, they may be stored at 2 to 8℃.

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

【REAGENT PREPARATION】

Bring all reagents and samples to room temperature before use.

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1×)

Pour entire contents (50 ml) of the **Washing Buffer (20×)** into a clean 1,000 ml graduated cylinder. Bring to final volume of 1,000 ml with pure or deionized water.

Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2 to 25℃. Washing Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (5 ml) of the **Assay Buffer (10×)** into a clean 100 ml graduated cylinder. Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.

Store at 2 to 8℃. Assay Buffer (1×) is stable for 30 days.

Detect Antibody

Mix well prior to making dilutions.

Make a **1：100** dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1×) in a clean plastic tube.

The diluted Detect Antibody should be used within 30 minutes after dilution.

Mouse Ig Positive Control

Reconstitute **Mouse Ig Positive Control** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization.

Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.

Sample Dilution: If your samples have high Ig content, Assay Buffer (1×) is used for dilution of cell culture medium, ascites and purified antibodies.

【ASSAY PROCEDURE】

Bring all reagents and samples to room temperature before use.

1) Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.

2) Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.

3) Add 300 µl Washing Buffer (1×) per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer (1×). Use the microwell strips immediately after washing. **Do not allow wells to dry.**

4) Add 100 µl of **Positive Control** to each well in plate column 1. Add 100 µl of Assay Buffer (1×) as negative control to each well in plate column 2.

5) Add 100 µl of sample with appropriate dilution to per well in each plate column.

6) Add 50 µl of diluted Detect Antibody to each well. Ensure reagent addition in step 4, 5 and 6 is uninterrupted and completed within 15 minutes.

7) Cover with an adhesive film. Incubate at room temperature (25℃±3℃) for 2 hours on a microplate shaker set at 300 rpm.

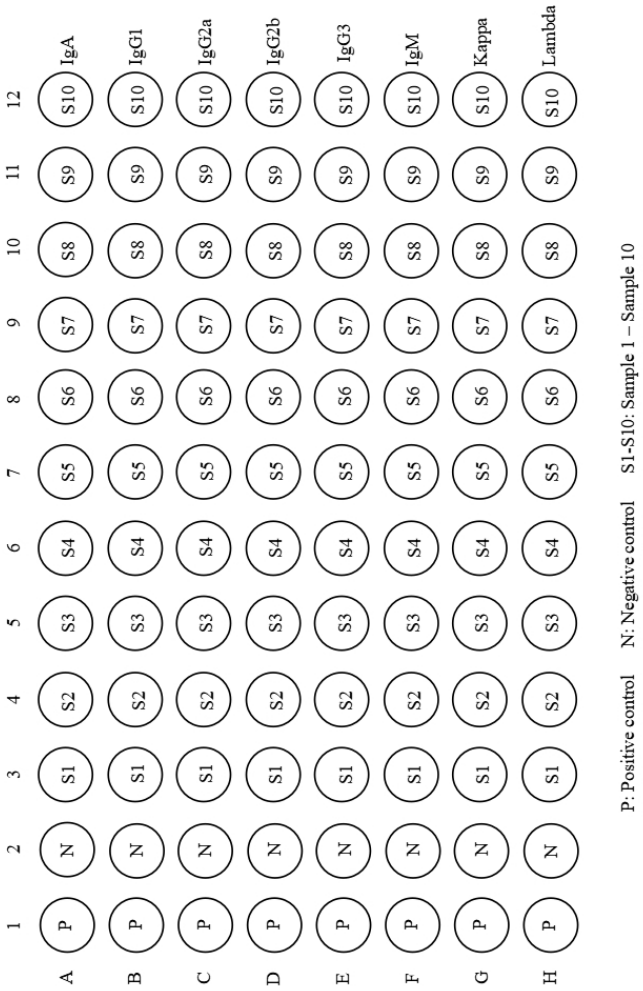
8) Aspirate each well and wash, repeating the process five times for a total six washes. Wash by filling each well with 300µl Washing Buffer (1×). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

9) Add 100 µl of Substrate Solution to each well. Incubate for 5 - 30 minutes at room temperature(25℃±3℃). **Protect from light.**

10) Add 100 µl of Stop Solution to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11) Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

【PLATE LAYOUT】



MULTI SCIENCES BIOTECH CO., LTD.

www.multisciences.net

E-mail: service@multisciences.net