BioOcean® Mouse Ig Isotyping ELISA Kit

Catalog Number: EK279
Size: 96 Test

For the quantitative determination of mouse Ig isotypes in hybridoma supernates, ascites and purified antibodies.

This package insert must be read entirely before using this product. For proper performance, follow the protocol provided with each individual kit.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Bio-Ocean LLC
3550 Lexington Ave, Suite 203, Shoreview, MN 55126
www.bioocean.com
Tel:1-800-852-7601
Fax:1-844-697-5634
Email:info@bioocean.com

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QUICK PROCEDURE FLOWCHART

1. **Prepare all reagents and positive control as directed.**

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

3. **Add 100 µl sample with appropriate dilution to per well in each plate column.**

4. **Add 50 µl diluted *Detect Antibody* to each well. Step 2, 3 and 4 should be completed within 15 minutes.**

5. **Incubate for 2 hour at RT.**

6. **Aspirate and wash 6 times.**

7. **Add 100 µl *Substrate Solution* to each well.**

Incubate for 5 - 30 minutes at RT. Protect from light.

8. **Add 100 µl *Stop Solution* to each well.**

9. **Read at 450 nm within 30 minutes. Correction 570 or 630 nm.**

DESCRIPTION

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

BioOcean Mouse Ig Isotyping ELISA Kit is based on the quantitative sandwich enzyme-linked immunosorbent assay technique to measure concentration of mouse Ig Isotyping in the samples. 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.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use expired kit or reagents.
- Do not use reagents from other lots or manufacturers. Do not prepare component by yourself.
- Disposable pipette tips, tubes or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- Bacterial or fungal contamination in either samples or reagents, or cross-contamination between reagents may cause erroneous results.

MATERIALS PROVIDED

Unopened kit should be stored at 2 - 8℃. 

- **Ig Isotyping Microplate** (1 plate): 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against mouse Ig Isotyping.
- **Ig Isotyping Detect Antibody** (1 vial, 80 µl): HRP-conjugate anti-mouse Ig Isotyping detect antibody; 100× liquid.
- **Positive Control**: mouse IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, kappa and lambda isotype control mix.
- **Assay Buffer (10×)** (1 bottle, 5 ml): PBS with 0.5 % Tween-20 and 5 % BSA.
- **Substrate** (1 bottle, 15 ml): TMB (tetramethyl-benzidine).
- **Stop Solution** (1 bottle, 15 ml): 0.18 M sulfuric acid.
- **Washing Buffer (20×)** (1 bottle, 50 ml): PBS with 1 % Tween-20.
- **Plate Covers** (5 strips).

STORAGE

Store kit reagents between 2 and 8℃. Immediately after use remaining reagents should be returned to cold storage (2 to 8℃). Expiry of the kit and reagents is stated on labels.
Expiration date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Unopened kit		Store at 2 - 8℃ (See expiration date on the label).
Opened/ Reconstituted Reagents	1× Washing Buffer 1× Assay Buffer Stop Solution Substrate TMB Detect Antibody	Up to 1 month at 2 - 8℃.
	Positive Control	Up to 1 month at ≤ -20℃ in a manual defrost freezer. Discard after use.
	Microplate Wells	Up to 1 month at 2 - 8℃. Return unused strips to the foil pouch containing the desiccant pack, reseal along entire edge to maintain plate integrity.

Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- **Microplate reader** capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
- **Pipettes and pipette tips**.
- 50 µl to 300 µl adjustable **multichannel micropipette** with disposable tips.
- Multichannel micropipette **reservoir**.
- **Beakers, flasks, cylinders** necessary for preparation of reagents.
- **Deionized or distilled water**.
- **Polypropylene** test tubes for dilution.

PRECAUTION

- Intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Treat all chemicals with caution because they can be potentially hazardous.
- It is recommended that this product is handled only by persons who have been trained in laboratory techniques and in accordance with the principles of good laboratory practice. Wear personal protection equipment such as laboratory coat, safety glasses and gloves.
- Avoid direct contact with skin or eyes. Wash immediately with water in the case of contact with skin or eyes. Avoid contact of skin or mucous membranes with kit reagents or specimens. See material safety data sheet(s) for specific advice.
- Pure water or deionized water must be used for reagent preparation.
- The Stop Solution provided with this kit is an acid solution. Wear personal protection equipment with caution.
- Do not expose kit reagents to strong light during storage and incubation.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- Use disposable pipette tips and/or pipettes to avoid microbial or cross-contamination of reagents or specimens which may invalidate the test.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the HRP and antibody conjugate.
- Substrate solution must be warmed to room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5℃.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

TECHNICAL HINTS

- All reagents including microplate, samples, positive control and working solution should be warmed to room temperature before use.
- To obtain accurate results, using adhesive film to seal the plate during incubation is suggested.
- Avoid foaming when mixing or reconstituting solutions containing protein.
- To avoid cross-contamination, use separate reservoirs for each reagent and change pipette tips between each positive control, sample and reagent.
- When using an automated plate washer, adding a 30 seconds soak period before washing step and/or rotating the plate between wash steps may improve assay precision.
- When pipetting reagents, maintain a consistent order of addition from well-to-well.
- Keep Substrate solution protected from direct strong light. Substrate Solution should turn to gradations of blue after a proper color development.
- Read absorbance within 30 minutes after adding stop solution.
- Take care not to scratch the inner surface of the microwells.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates – Remove particulates by centrifugation and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

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

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 1000 ml graduated cylinder.
Bring to final volume of 1000 ml with distilled 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 as needed.
The diluted Detect Antibody should be used within 30 minutes after dilution.

Sample Dilution

If your samples need to be diluted, Assay Buffer (1×) is used for dilution of cell culture medium, ascites and purified antibodies.

Mouse Ig Positive Control

Reconstitute Mouse Ig Positive Control by addition of distilled water. Reconstitution volume is stated on the label of the positive control vial. Swirl or mix gently to insure complete and homogeneous solubilization.
Allow the positive control to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents including microplate, samples, positive control 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. Seal the plate with an *adhesive film*. Incubate at room temperature (18 to 25℃) for 3 hours on a microplate shaker set at 300 rpm.
8. Aspirate each well and wash by filling each well with 300 µl *Washing Buffer (1×)*, repeat five times for a total six washes. Complete removal of liquid at each step is essential to the best performance. After the last wash, remove any remaining *Washing Buffer (1×)* by aspirating or decanting. Invert the plate and tap it against clean paper towels.
9. Add 100 µl of *Substrate Solution* to each well. Incubate for 5 - 30 minutes at room temperature. 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