Mouse Immunoglobulin Isotyping

ELISA Kit

Cat #: orb1289380 (manual)

Sandwich Enzyme-Linked Immunosorbent Assay for the Qualitative Detection of Mouse Ig Isotypes.

For research use only. Not for diagnostic or therapeutic procedures.

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INTRODUCTION

The Mouse Immunoglobulin Isotyping ELISA Kit enables rapid, efficient identification of mouse immunoglobulin isotypes. This kit employs a direct horseradish peroxidase-labeled system. These features lead to a significant reduction in assay time without sacrificing sensitivity. Each kit supplies: 6 mouse immunoglobulin isotype-specific monoclonal antibodies conjugated horseradish peroxidase (HRP) (IgG1, IgG2a, IgG2b, IgG3, IgM, IgA), TMB substrate solutions, stop solutions, sample diluent buffers, positive control and negative control.

ASSAY PRINCIPLES

The Mouse Immunoglobulin Isotyping ELISA Kit is an in vitro enzyme-linked immunosorbent assay for the qualitative measurement of mouse immunoglobulin isotyping in cell culture supernates, ascites, purified antibodies preparations. This assay employs an antibody specific for mouse immunoglobulin coated on a 96-well plate. Samples are pipetted into the wells and mouse immunoglobulin in a sample is bound to the wells by the immobilized antibody. The wells are washed and immunoglobulin isotype-specific monoclonal antibodies conjugated horseradish peroxidase are added. After washing away unbound antibody, a TMB substrate solution is added to the wells and color develops. The stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

KIT COMPONENTS

Component	Volume
96-well Plate Coated with Anti-Mouse Immunoglobulin Antibody	12 x 8 Strips
HRP-Labeled Anti-mouse IgG1 Antibody	1.6 ml
HRP-Labeled Anti-mouse IgG2a Antibody	1.6 ml
HRP-Labeled Anti-mouse IgG2b Antibody	1.6 ml
HRP-Labeled Anti-mouse IgG3 Antibody	1.6 ml
HRP-Labeled Anti-mouse IgM Antibody	1.6 ml
HRP-Labeled Anti-mouse IgA Antibody	1.6 ml
Positive Control	1.2 ml
Negative Control	1.2 ml
Sample Diluent	15 ml
Wash Buffer (20X)	30 ml
TMB Substrate Solution	12 ml
Stop Solution	12 ml
Plate Adhesive Strips	3 Strips
Technical Manual	1 Manual

STORAGE AND STABILITY

All kit components are stable at 2 to 8 °C. Opened Microplate Wells or reagents may be stored for up to 1 month at 2 to 8 °C. Return unused wells to the pouch containing the desiccant pack, and reseal along the entire edge.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and pipette tips to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1-liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

HEALTH AND SAFETY PRECAUTIONS

1. Reagents provided in this kit may be harmful if ingested, inhaled, or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.
2. Stop Solution contains 2 N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand, and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution before disposing of the plate.

REAGENT PREPARATION

1. Sample Preparation

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

Cell culture supernates: Remove particulates by centrifugation, assay immediately or dilute with sample diluent (1:1), aliquot and store samples at -20°C.

Ascites: Remove particulates by centrifugation, dilute with sample diluent (e.g. 1:50000), aliquot and store samples at -20°C. *Note: the ascites composition is very complicated, the result may be affected.*

2. Wash Buffer Working Solution Preparation

Pour entire contents (30 ml) of the Wash Buffer Concentrate into a clean 1,000 ml graduated cylinder. Bring the final volume to 600 ml with glass-distilled or deionized water (1:20).

ASSAY PROCEDURE

All reagents must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly.

1. Add each sample, positive control, negative control into 6 wells, 100 µl per well.

2. Cover well and incubate for 60 minutes at room temperature.

3. Remove the cover, discard the solution, and wash the plate 3 times with Wash Buffer Working Solution, and each time let Wash Buffer Working Solution stay in the wells for 1 - 2 minutes. Blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

4. Add 6 kinds of HRP-Labeled detection antibody into 6 wells of each sample and incubate the plate at 37°C for 30 minutes.

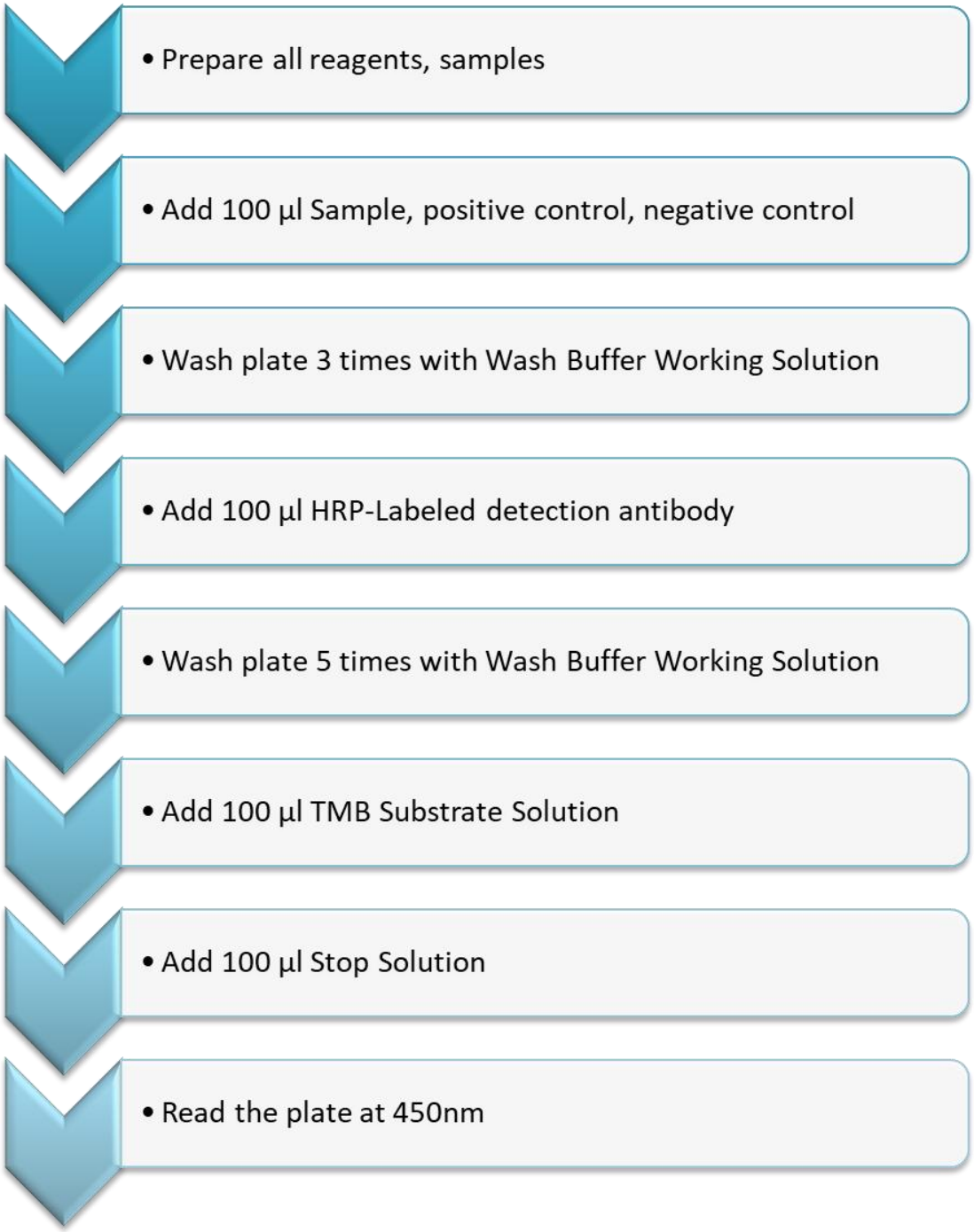
5. Wash the plate 5 times with Wash Buffer Working Solution, and each time let the wash buffer stay in the wells for 1 - 2 minutes. Discard the wash buffer and blot the plate onto paper towels or other absorbent material.

6. Add 100 µl of TMB Substrate Solution into each well and incubate the plate at 37°C in the dark for 20 minutes.

7. Add 100 µl of Stop Solution into each well. The color changes into yellow immediately.

8. Read the O.D. absorbance at 450nm in a microplate reader within 30 minutes after adding the Stop Solution.

ASSAY PROCEDURE SUMMARY



SUGGESTED PLATE LAYOUT

The following figure depicts a scheme of the arrangement of samples (S), negative controls (N), and positive controls (P).

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