Introduction

Zymed's Mouse MonoAb ID/SP Kit is a complete set of ELISA reagents primarily designed to determine the class and subclass of mouse monoclonal antibodies in culture supernatant.

Advantages of Streptavidin-Biotin Amplification System

High sensitivity and low background.
Short incubation time.

Principles of the Assay

This assay is based on a streptavidin-biotin amplification system. Affinity purified anti-mouse class and subclass antibodies conjugated with biotin are employed in isotyping mouse immunoglobulins secreted in culture supernatant. Streptavidin, which binds exceptionally well ($K_d=10^{-15} \text{ M}$) to biotin, is coupled to horseradish peroxidase to serve as the signal generating reagent.

Capacity and Sensitivity

These reagents are sufficient for isotyping 100 mouse supernatants. The sensitivity of this assay is approximately 50 ng.

Intended Use

This kit can only be used in an antigen dependent ELISA. These reagents will not perform in an antigen-independent assay.

Storage

The kit should be stored at 4°C.

Note

Multi-well type plastic plates are recommended for use with this kit. (e.g. microtiter plates with capacities of 0.25 ml per well). Other types of solid-phase matrix for coating antigens can also be used, however, the amount of reagents required for the assay may be altered depending upon the dimensions and the nature of the solid matrix.

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**Kit Contents**

Kit Contents (A -I) are prediluted in PBS, pH 7.4, containing 1% bovine serum albumin, and 0.05% sodium azide and are ready to use.

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Biotinylated anti-Mouse IgG1 (γ1 chain specific) -</td>
<td>6 ml</td>
</tr>
<tr>
<td>B</td>
<td>Biotinylated anti-Mouse IgG2a (γ2a chain specific) -</td>
<td>6 ml</td>
</tr>
<tr>
<td>C</td>
<td>Biotinylated anti-Mouse IgG2b (γ2b chain specific) -</td>
<td>6 ml</td>
</tr>
<tr>
<td>D</td>
<td>Biotinylated anti-Mouse IgG3 (γ3 chain specific) -</td>
<td>6 ml</td>
</tr>
<tr>
<td>E</td>
<td>Biotinylated anti-Mouse IgA (α chain specific) -</td>
<td>6 ml</td>
</tr>
<tr>
<td>F</td>
<td>Biotinylated anti-Mouse IgM (μ chain specific) -</td>
<td>6 ml</td>
</tr>
<tr>
<td>G</td>
<td>Biotinylated anti-Mouse κ light chain -</td>
<td>6 ml</td>
</tr>
<tr>
<td>H</td>
<td>Biotinylated anti-Mouse λ light chain -</td>
<td>6 ml</td>
</tr>
<tr>
<td>I</td>
<td>Biotinylated Antibody Control (Negative Control) (Diluted biotinylated normal immunoglobulin) -</td>
<td>6 ml</td>
</tr>
<tr>
<td>J</td>
<td>Substrate Buffer, Concentrated (10x) - (1 M citrate, pH 4.2, containing 0.3% H2O2)</td>
<td>10 ml</td>
</tr>
<tr>
<td>K</td>
<td>ABTS Substrate, Concentrated (50x) - (2,2-azino-di[3-ethylbenzthiazoline sulfonic acid])</td>
<td>2 ml</td>
</tr>
<tr>
<td>L</td>
<td>Blocking Solution, Concentrated (50x) - (25% BSA in PBS and 0.05% NaN3)</td>
<td>2.5 ml</td>
</tr>
<tr>
<td>M</td>
<td>HRP-Streptavidin, Concentrated (50x) -</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>N</td>
<td>50% Tween 20 -</td>
<td>2.5 ml</td>
</tr>
</tbody>
</table>
Preparation of Reagents

1) PBS-Tween - Prepare 50 mM phosphate buffered saline (PBS), pH 7.4 (not provided). Add one drop of 50% Tween 20 to every 50 ml of buffer.

2) HRP-Streptavidin - Add one drop of concentrated conjugate to every 2.5 ml of PBS-Tween.

3) Working Substrate Solution - Prepare fresh before use. Add one drop of ABTS concentrate and 5 drops of substrate buffer concentrate to every 2.5 ml of distilled water. Discard the unused portion.

4) Blocking Solution - Add one drop of blocking solution to every 2.5 ml of PBS (no Tween).

Preassay Procedure

Before running assay prepare an antigen coated plate. Dilute your antigen to 10 µg/ml (or to your optimized concentration) and add 50 µl to all wells. Incubate at 4°C overnight, or for 2 hours at room temperature. Decant and slap onto a paper towel until dry. Add 200 ul of 1% BSA to all wells. Incubate at 37°C for 1 hour. Decant and slap onto a paper towel until dry. (Microwell plate, and antigen not provided.)

If the antigen that you are coating contains endogenous peroxidase activity you will need to treat your coated plate further. Two methods are described below. Depending on the antigen you are using, one method may work better than the other.

(1) Method 1: Add 150 µl of 0.1% phenylhydrazine in PBS (no Tween) to each well of your antigen-coated plate. Incubate for 1 hour at 37°C.

(2) Method 2: Add 150 µl of 0.3% hydrogen peroxide in methanol to each well of your antigen-coated plate. Incubate for 30 minutes at room temperature.
**Procedure: Antigen Dependent Protocol for Isotyping Mouse Monoclonal Antibodies**

1) Add 50 µl of your first sample to each well in the first row of your antigen-coated plate. Add one additional sample to each subsequent row. Up to 8 individual samples may be isotyped per microtiter plate.

2) Incubate at 37°C for 30 minutes. Wash 4 times with PBS-Tween.

3) Add 50 µl of buffer to each well of the first column. This will serve as your blank column. Add 1 drop of biotinylated antibody control to each well of the second column. This will serve as your negative control column. Add 1 drop of subclass specific biotinylated anti-Mouse IgG1 to each well of the third column. Add the rest of the subclass specific antibodies to columns 4 through 10 as was done for biotinylated anti-Mouse IgG1.

4) Incubate at 37°C for 30 minutes. Wash 4 times with PBS-Tween.

5) Add 50 µl of diluted HRP-Streptavidin to all wells.

6) Incubate at 37°C for 30 minutes. Wash 4 times with PBS-Tween.

7) Add 100 µl of working substrate solution to all wells.

8) Incubate at room temperature and monitor for 30 minutes.

9) Read positive results either qualitatively by visual inspection or quantitatively with a spectrophotometer at 405 nm. The plates can then be sealed with transparent adhesive tape and photographed for permanent records.

**Remarks**

For research use only. Not for human use or drug use. These reagents contain either sodium azide or proclin as preservatives.

**Conditions**

Nothing disclosed herein is to be construed as a recommendation to use our products in violation of any patents. We cannot be responsible for patent infringement or other violations that may occur with the use of our products.

**References for blocking endogenous peroxidase activity**


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