Procedure 2: Antigen Dependent Protocol for Isotyping Mouse Monoclonal Antibodies

1) Follow Preassay Procedure.
2) 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.
3) Incubate at 37°C for 30 minutes. Wash 4 times with washing buffer.
4) 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.
5) Incubate at room temperature for 15 minutes. Wash 4 times with washing buffer.
6) Add 50 µl of diluted HRP-Streptavidin to all wells.
7) Incubate at room temperature for 15 minutes. Wash 4 times with washing buffer.
8) Add 100 µl of working substrate solution to all wells.
9) Incubate at room temperature and monitor for 10 minutes.
10) 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.

Storage The kit should be stored at 4°C.

Remarks

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

References

1. 97-6550 Mouse Screening/Isotyping Kit -Xiang, J. et al; (1995) Autologous Human B-Cell Immune Response to Pulmonary Adenocarcinomatous Polymorphic Epithelial Mucin. *J. of Clin. Immunol.* 15(2):74.
2. Strauss, W. (1979) *J. Histochem. Cytochem.,* 27:1349.
3. Streefkerk, J.G. (1972) *J. Histochem. Cytochem.,* 20:829.

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PI976550 (Rev 10/08) DCC-08-1089

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97-6550 1 Kit
Mouse Screening/
Isotyping Kit

Mouse Immunoglobulin Screening/Isotyping Kit

Introduction

Invitrogen 's Screening/Isotyping Kit for mouse antibodies contains a complete set of ELISA reagents primarily designed for screening, and determining the class and subclass of mouse monoclonal antibodies in culture supernatant.

Advantages of Streptavidin-Biotin Amplification (LAB-SA) System

High sensitivity.
Low background.
Short incubation time.

Principles of the Assay

This assay is based on a LAB-SA system. Biotinylated, affinity-purified antibodies (anti-mouse IgG+IgA+IgM (H+L), anti-mouse class, and anti-subclass antibodies) are employed in screening and isotyping mouse immunoglobulins secreted in culture supernatant. Streptavidin, which binds exceptionally well ($K_d=10^{-15}$ M) to biotin, is coupled to horseradish peroxidase to serve as the signal generating reagent.

Capacity and Sensitivity

These reagents are sufficient for screening 10,000, and 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.**

Note: Multi-well type plastic plates are recommended for use with this kit. (e.g. microtiter plates with capacities of 0.25ml 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 (BSA), and 0.05% Sodium Azide and are **ready-to-use**.

A)	Biotinylated anti-Mouse IgG1 -	6 ml
B)	Biotinylated anti-Mouse IgG2a -	6 ml
C)	Biotinylated anti-Mouse IgG2b -	6 ml
D)	Biotinylated anti-Mouse IgG3 -	6 ml
E)	Biotinylated anti-Mouse IgA -	6 ml
F)	Biotinylated anti-Mouse IgM -	6 ml
G)	Biotinylated anti-Mouse kappa light chain -	6 ml
H)	Biotinylated anti-Mouse lambda light chain -	6 ml
I)	Biotinylated Antibody Control (Negative Control) - (Diluted biotinylated normal immunoglubulin)	6 ml
J)	Biotinylated anti-Mouse IgG+IgA+IgM (H+L) Conc. (100x), (Antibody is supplied in PBS, pH 7.4, with 1% BSA & 0.05% NaN_3)	2 Bottles, 3 ml
K)	ABTS Substrate, Concentrated (100x) - (2,2-azino-di[3-ethylbenzthiazoline sulfonic acid])	15 ml
L)	Blocking Solution, Concentrated - (30% BSA in PBS and 0.05% NaN_3)	3.5 ml
M)	HRP-Streptavidin, Concentrated (100x) - (2 Bottles)	1.5 ml
N)	Tween 20, Concentrated (1000x) -	15 ml
O)	Hydrogen Peroxide, Concentrated (1000x) -	2 ml
P)	Citrate Buffered (Powder) -	3 Bags
Q)	Phosphate Buffered Saline (Powder) -	12 Bags

Preparation of Reagents

1) Washing Buffer/Diluent - 0.05% Tween 20 in 50 mM Phosphate Buffered Saline (PBS), pH 7.4 - Dissolve one bag of PBS powder (Q) in 1 L of distilled water. Add 20 drops of Tween 20 (N).
2) Substrate Buffer - Dissolve one bag of Citrate Buffer powder (P) in 500 ml of distilled water. Add 10 drops of hydrogen peroxide (O). This will make 0.03% H_2O_2 in 100 mM Citrate Buffer, pH 4.2.
3) HRP-Streptavidin - Add one drop of concentrated conjugate (M) to every 5 ml of Diluent (step 1).
4) Working Substrate Solution - Prepare fresh before use. Add one drop of ABTS concentrate (K) to every 5 ml of substrate buffer (step 2). Note: ABTS may show precipitation at 4°C. It will dissolve completely when warmed to room temperature.
5) Blocking Solution - Add 3 drops of blocking solution (L) to every 5 ml of PBS (follow step 1 but do not add Tween). (If BSA cannot be employed use 5% non fat dry milk (Carnation Brand) in PBS.)

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6) Add one drop of concentrated biotinylated anti-mouse IgG+IgA+IgM (H+L) antibody (J) to every 5 ml of Diluent (step 1).

Preassay Procedure

Before running the assay prepare an antigen coated plate. Dilute your antigen to 10µg/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 to remove liquid. Add 200 µl of 1% BSA to all wells. Incubate at 37°C for 1 hour. Decant and slap onto a paper towel to remove liquid. (Microtiter plate and antigen are 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 1: Screening Protocol

1) Add 50 µl of culture supernatants to each well of your antigen coated plate.
2) Incubate at 37°C for 30 min. Decant supernatants.
3) Add 50 µl of diluted biotinylated anti-mouse IgG+IgA+IgM (H+L) antibody to each every well.
4) Incubate at room temperature for 15 min. Flick out biotinylated antibody.
5) Add 50 µl of diluted HRP-Streptavidin to every well.
6) Incubate at room temperature for 15 min. Wash 4 times with washing buffer.
7) Add 100 µl of working substrate solution to every well.
8) Incubate at room temperature and monitor for 10 min.
9) Read positive results either qualitatively by visual inspection or quantitatively with a spectrophotometer at 415 nm (405 nm filter is also acceptable). The plates can then be sealed with transparent adhesive tape and photographed for permanent records.

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