INSTRUCTIONS

PIERCE

ImmunoPure® Monoclonal Antibody Isotyping Kit I(HRP/ABTS)

3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

37501 0302w

Introduction

Pierce has developed the ImmunoPure® Monoclonal Antibody Isotyping Kits to allow the researcher to quickly and easily screen and determine classes and subclasses for mouse monoclonal antibodies in the supernatants of hybridoma or myeloma cultures. This kit is not designed for use with ascites or serum. The kits are designed for an enzyme immunoassay format and contain sufficient reagents for the isotyping of approximately 100 mouse monoclonal antibodies (1,000 wells). Antigen-dependent or antigen-independent techniques can be employed.

Product Description

Number	Description
37501	**ImmunoPure® Monoclonal Antibody Isotyping Kit I (HRP/ABTS).**

The kit is stable for 6 months at 4°C.

Contents:

Each reagent is provided in a dropper bottle.

1. 6 ml each of the following are supplied. The antibodies are prepared in rabbits and suppliedin phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 0.05% sodium azide.
   a. Normal Rabbit Serum (negative control)
   b. Anti-Mouse IgG1 (gamma 1 chain specific)
   c. Anti-Mouse IgG2a (gamma 2a chain specific)
   d. Anti-Mouse IgG2b (gamma 2b chain specific)
   e. Anti-Mouse IgG3 (gamma 3 chain specific)
   f. Anti-Mouse IgA (alpha chain specific)
   g. Anti-Mouse IgM (mu chain specific)
   h. Anti-Mouse Kappa Light Chain
   i. Anti-Mouse Lambda Light Chain
2. Horseradish Peroxidase Conjugated Goat Anti-Rabbit IgG, 1.5 ml
3. ABTS Substrate Buffer Concentrate (10X). The composition is 1 M citrate, pH 4.2, with 0.3% hydrogen peroxide, 10 ml
4. ABTS Substrate (50X) (ABTS, 2, 2-azino-di[3-ethyl benzthiazoline sulfonic acid]), 2 ml
5. Tween® 20 (50%), 2.5 ml
6. Coating Antibody. This is a goat anti-mouse Ig(G + A + M) and is supplied at a 0.5 mg/ml concentration in PBS, pH 7.4, containing 10% glycerol and 0.05% sodium azide, 2.5 ml
7. Blocking Solution Concentrate (50X). The composition of this buffer is 25% bovine serum albumin in phosphate buffered saline containing 0.05% sodium azide, 2.5 ml
8. Positive Control. This is a monoclonal mouse IgG1 in RPMI-1640 tissue culture medium with 10% fetal bovine serum and 0.05% sodium azide, 1 ml

Protocol for Isotype Determination of Mouse Monoclonal Antibodies by Antigen-Dependent Technique

In this protocol a total of 9 microtiter plate wells are used to screen and determine the class/subclass of one mouse monoclonal antibody. Eight wells contain the experimentals and one well is a negative control. The dilutions given for the kit components provide enough reagent for isotyping two mouse monoclonal antibodies.

Preparation of Reagents

A. Phosphate Buffered Saline (PBS). 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.

B. Wash Buffer. Add 1 drop of 50% Tween® 20 (supplied), 50 ml of PBS.

C. Horseradish Peroxidase Conjugated Goat Anti-Rabbit IgG working solution. Add 1 drop of HRP concentrated Goat Anti-Rabbit IgG (supplied) to 2.5 ml of Wash Buffer (prepared immediately before use).

D. 1X ABTS substrate buffer. Add 5 drops of the 10X ABTS Substrate Buffer concentrate (supplied) to 2.5 ml of distilled water.

E. 1X ABTS substrate solution. Add one drop of 50X ABTS Substrate (supplied) to 2.5 ml of 1X ABTS substrate buffer (prepare immediately before to use).

F. Coating Buffer. 0.1 *M* sodium bicarbonate, pH 9.5. Prepare by adding 70 ml of 0.1 M NaHCO3 to 30 ml of 0.1 M Na_2CO_3 to obtain a pH of approximately 9.5.

G. Antigen Solution. 1-10 µg/ml in coating buffer.

H. 1X Blocking Solution. Add one drop of 50X Blocking solution (supplied) to 2.5 ml of PBS.

I. Class/subclass specific rabbit anti-mouse immunoglobulins (supplied).

J. Normal Rabbit Serum (supplied).

Method

1. Coat the wells of the plate with antigen by placing 50 µl of the antigen solution into each of 9 separate wells and incubating at 4°C overnight or at room temperature for 2 hours.

   Note: If the antigen being coated contains endogenous peroxidase activity (such as cells), the antigen-coated wells must be treated to eliminate this activity. This can be accomplished by adding 150 µl of 0.1% phenylhydrazide in PBS to each antigen-coated well and incubating for 1 hour at 37°C. In some cases, however, this treatment will be insufficient to completely eliminate all endogenous peroxidase activity. In these cases, an additional second treatment step is required with methanol and hydrogen peroxide: from a solution composed of 99 ml methanol and 1 ml of 30% hydrogen peroxide, add 150 µl to each antigen-coated well and incubate for 30 minutes at room temperature.

2. Remove the above solution and block by adding 125 µl of the 1X Blocking solution and incubating at 37°C for 1 hour.

3. Remove the solution and wash 4X 125 µl with Wash Buffer.

4. Add 50 µl of culture supernatant to each antigen-coated well on the plate.

5. Incubate at 37°C for 1 hour.

6. Remove the solution and wash 4X 125 µl with Wash Buffer.

7. Separately add one drop (50 µl) of Normal Rabbit Serum or subclass-specific anti-mouse immunoglobulins to each antigen-coated well on the plate. The Normal Rabbit Serum serves as a negative control. Record additions using the template at the end of this booklet.

8. Incubate at 37°C for 1 hour.

9. Remove the solution and wash 4X 125 µl with Wash Buffer.

10. Add 50 µl of Horseradish Peroxidase Conjugated Goat anti-Rabbit IgG working solution to each antigen-coated well on the plate.

11. Incubate at 37°C for 1 hour.
12. Remove the solution and wash 4X 125 µl with Wash Buffer.
13. Add 100 µl of 1X ABTS substrate solution to each antigen-coated well on the plate.
14. Incubate at room temperature and monitor color development for approximately 30 minutes.
15. Read results either qualitatively by visual inspection or quantitatively with a spectrophotometer at 405 nm. The positive wells should have an optimum absorbance ranging from 0.8 to 1.2. The plates can then be sealed with transparent adhesive tapes and photographed for permanent record.

Protocol for Isotype Determination of Mouse Monoclonal Antibodies by Antigen-Independent Technique

In this protocol, a total of 10 microtiter plate wells are used to screen and determine the class/subclass of one mouse monoclonal antibody. Eight wells contain the experimentals. A negative and positive control are included in two separate wells. The dilutions given for the kit components provide enough reagent for isotyping two mouse monoclonal antibodies.

Materials

A. Phosphate Buffered Saline (PBS). 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.

B. Wash Buffer: Add 1 drop of 50% Tween® 20 (supplied) to 50 ml of PBS.

C. Coating Buffer: 0.1 M sodium bicarbonate, pH 9.5. Prepare by adding 70 ml of 0.1 M $NaHCO_3$ to 30 ml of 0.1 M Na_2CO_3 to obtain a pH of approximately 9.5.

D. Coating Antibody Working Solution: Add 1 drop of coating antibody solution to 5 ml of Coating Buffer (prepare immediately before use).

E. Positive Control: (supplied).

F. 1X Blocking Solution. Add 1 drop of 50X Blocking Solution (supplied) to 2.5 ml of PBS.

G. Horseradish Peroxidase Conjugated Goat Anti-Rabbit IgG working solution. Add 1 drop of HRP conjugated Goat Anti-Rabbit IgG to 2.5 ml of Wash Buffer.

H. 1X ABTS Substrate Buffer: Add 5 drops of 10X ABTS Substrate Buffer Concentrate (supplied) to 2.5 ml of distilled water.

I. 1X ABTS Substrate Solution. Just prior to use, add 1 drop of 50X ABTS Substrate (supplied) to 2.5 ml of 1X ABTS substrate buffer.

J. Class/subclass specific rabbit anti-mouse immunoglobulins (supplied).

K Normal Rabbit Serum (supplied).

Method

1. Coat the wells of the plate with coating antibody working solution by placing 50 µl of the solution into each of 10 separate wells and incubating at 4°C overnight or at room temperature for 2 hours. Identify these wells as #1 - #10.
2. Remove the solution and block by adding 125 µl of the 1X Blocking Solution and incubating at 37°C for 1 hour.
3. Remove the solution and wash 4X 125 µl with Wash Buffer.
4. Remove the solution and add 50 µl of culture supernatant to each of 9 separate wells (well #1 - #9) of the plate.

   Note: Add 1 drop (50 µl) of the Positive Control to a separate well (well #10).
5. Incubate at 37°C for 1 hour.
6. Remove the solution and wash 4X 125 µl with Wash Buffer.

7. Separately add one drop (50 µl) of Normal Rabbit Serum or subclass-specific anti-mouse immunoglobulins to each of 9 separate wells on the plate (well #1 - #9). Separately add one drop of Anti-Mouse IgG1 to well #10. Record additions using the template at the end of this booklet.
8. Incubate at 37°C for 1 hour.
9. Remove the solution and wash 4X 125 µl with Wash Buffer.
10. Add 50 µl of Horseradish Peroxidase Conjugated Goat Anti-Rabbit IgG working solution to each of the 10 wells.
11. Incubate at 37°C for 1 hour.
12. Remove the solution and wash 4X 125 µl with Wash Buffer.
13. Add 100 µl of 1X ABTS Substrate Solution to each of the 10 wells.
14. Incubate at room temperature and monitor color development for approximately 30 minutes.
15. Read results either qualitatively by visual inspection or quantitatively with a spectrophotometer at 405 nm. The positive wells should have an optimum absorbance ranging from 0.8 to 1.2. The plates can then be sealed with transparent adhesive tapes and photographed for permanent records.

Triton is a registered trademark of Rohm and Haas.
Tween is a registered trademark of ICI Americas.
©Copyright Pierce Chemical Company, 12/2000, Printed in U.S.A.

Name ____ **Date**______________

Experiment ____

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												