P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 – Tel. (435) 755-9848 - Fax (435) 755-0015 - www.scytek.com

Mouse to Mouse Alk-Phos (Permanent Red) Staining System

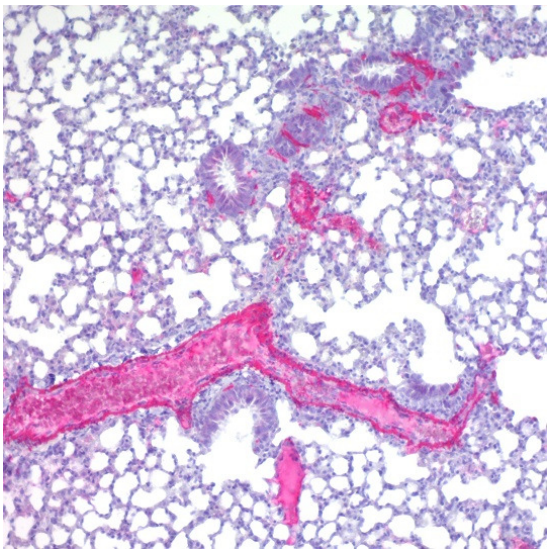
Description: The Mouse to Mouse staining kit provides unmatched sensitivity with incubation times of 10 minutes each for the Link Antibody and Enzyme Label. This kit includes our Permanent Red chromogen, which can be coverslipped with solvent based mounting media for long term storage.

Species of Origin: Goat
Antigen Specificity: Anti-Polyvalent (Mouse, Rat, Rabbit and Guinea Pig)

Uses/Limitations:
Not to be taken internally.
For In Vitro Diagnostic Use.
Histological applications.
Do not use if reagents become cloudy.
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile.

Control Tissue:
Any well-fixed tissue section.
Frozen tissue section.
Cytocentrifuge preparation.

Ordering Information and Current Pricing at www.scytek.com



Mouse lung stained with Smooth Muscle Actin; Clone 1A4. Magnification 100X.

Test Capacity: 70 Slides

Kit Contents:

Item #	Description	Volume
AAA008	Super Block	8 ml
MTM008	Mouse-to-Mouse Blocking Reagent	8 ml
ABN008	UltraTek Anti-Polyvalent	8 ml
ABM008	UltraTek Alk-Phos	8 ml
PRC002	Permanent Red Concentrate	2 ml
PRB005	Permanent Red Buffer	5 ml x 7 vials

Recommended, But Not Included:

Item #	Description
CPL500	Citrate Plus
HAQ500	Hematoxylin for Automation
BRT500	Bluing Reagent

Storage: 2° C 8° C

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Storage: Store at 2-8°C.

Precautions: Avoid contact with skin and eyes.
Harmful if swallowed.
Follow all Federal, State, and local regulations regarding disposal.

Procedure:

1. Deparaffinize and rehydrate tissue section.
2. Wash 2 times in buffer.
3. If required, incubate tissue in digestive enzyme.
4. Wash 4 times in buffer.
5. Apply Super Block (blue cap), and incubate for 5 minutes at room temperature to block nonspecific background staining. **Note:** Do not exceed 10 minutes or there may be a reduction in desired stain.
6. Wash 1 time in buffer.
7. Apply Mouse to Mouse Block and incubate10-60 minutes. Incubation time is dependent on the amount of endogenous Ig found in the tissue.
8. Wash 4 times in buffer.
9. Apply primary antibody and incubate according to manufacturer's protocol.
10. Wash 4 times in buffer.
11. Apply UltraTek Anti-Polyvalent (yellow cap), and incubate for 10 minutes at room temperature.
12. Wash 4 times in buffer.
13. Apply UltraTek Alk-Phos (red cap), and incubate for 10 minutes at room temperature.
14. Rinse 4 times in buffer.
15. Rinse 1 time in Distilled/DI water.

Mix Permanent Red Concentrate with Permanent Red Buffer.

16. Add 2 drops (60ul) Permanent Red Chromogen (PRC002) to each 5ml vial of Permanent Red Buffer (PRB005), mix by swirling and apply to tissue for 5 minutes.
17. Rinse 1 time in Distilled/DI Water.
18. Apply Permanent Red Chromogen/Substrate mixture and incubate for a second 5 minute period.
19. Rinse 1 time in Distilled/DI water.
20. Counterstain as desired.
21. Dehydrate slides and clear in xylene or xylene substitute.
22. Coverslip using a permanent mounting media.

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Troubleshooting Guide

Overstaining:

1. Concentration of the primary antibody was too high or the incubation time was too long.
2. Temperature during incubation was too high.
3. Incubation time with link antibody or streptavidin/enzyme label was too long.

Nonspecific Background Staining:

1. Rinsing between steps was inadequate.
2. Tissue was allowed to dry with reagents on.
3. Folds in tissue trapped reagents.
4. Tissue contains endogenous alkaline phosphatase.
5. Tissue contains endogenous biotin.
6. Antigen migrated in tissue.
7. Excessive tissue adhesive on slides.
8. Inadequate blocking with protein block.
9. Inadequate blocking with Mouse-To-Mouse Block.

Weak Staining:

1. Primary antibody concentration was too low or incubation time was too short.
2. Reagents are past their expiration date.
3. Inadequate removal of wash water between steps, resulting in dilution of reagents.
4. Counterstain or mounting media were incompatible and dissolved the chromogen reaction product.
5. Room temperature was excessively cool.
6. The primary antibody does not recognize an antigen that survives fixation and embedding in high enough amounts.
7. Excessive incubation with protein block (Super Block).

No Staining:

1. Steps were inadvertently left out.
2. There is no antigen in the tissue.
3. The primary antibody is not of mouse, rat, rabbit or guinea pig origin.

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