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

PolyTek Anti-Mouse Polymerized Alk-Phos

Description:

PolyTek Anti-Mouse Polymerized Alk-Phos has been developed to provide the cleanest, most consistent staining available. Developed in the research laboratories of ScyTek, the system is based on a polymerized alkaline phosphatase label that eliminates biotin and its' associated background issues from the equation. In addition, this product reduces the steps required for immunohistochemical staining by combining two steps from the traditional Biotin-Streptavidin system. PolyTek Anti-Mouse Polymerized Alk-Phos is effective with antibodies of mouse or rat origin.

Species of Origin:	Goat
Antigen Specificity:	Anti-Mouse
Preadsorbed Against:	Human
Enzyme Conjugate:	Alkaline Phosphatase

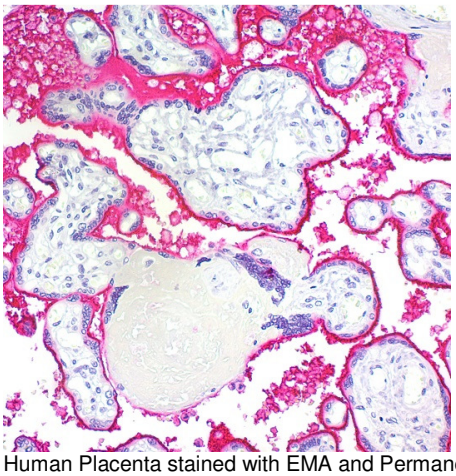
Uses/Limitations:

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



Human Placenta stained with EMA and Permanent Red Chromogen. Magnification 200X

Availability:

Item #	Volume
PAT008	8 ml
PAT015	15 ml
PAT125	125 ml
PAT500	500 ml
PAT999	1000 ml

Recommended, But Not Included:

Item #	Description
CPL500	Citrate Plus
AAA500	Super Block
PRD500	Permanent Red Bulk Pack (For Alkaline Phosphatase)
HMM500	Hematoxylin, Mayer's (Lillie's Modification)
BRT500	Bluing Reagent

Storage:

Store at 2-8℃.

Storage: 2° C 8° C

ScyTek Laboratories, Inc.
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Prinsessegracht 20
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Precautions: Avoid contact with skin and eyes.
Harmful if swallowed.
Follow all Federal, State, and local regulations regarding disposal.

Procedure:

1. Rehydrate tissue slides.
2. In a glass or plastic (Autoclavable) Coplin jar, add 5 ml of Citrate Plus (CPL) and 45 ml of deionized water.
3. Submerge slides in diluted Citrate Plus and loosely cap.
4. Add Distilled water to bottom of Autoclave or Pressure Cooker (about 1 inch deep in Pressure Cooker).
5. Place Coplin jar in Pressure Cooker or Autoclave.
6. Turn heat on and allow pressure to rise to 20-25 PSI.
7. Maintain pressure at 20-25 PSI for 5 minutes.
8. Turn off heat source and allow to cool.
9. When pressure has dropped to ambient, carefully remove lid or open door.
10. Using tongs, remove Coplin Jar and place on counter.
11. Once Coplin Jar cools to room temperature remove slides, rinse several times in buffer and proceed with staining as usual.
12. Rinse in distilled/DI water.
13. Rinse 3 times in buffer.
14. Apply Super Block (AAA), 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.
15. Rinse 1 time in buffer.
16. Apply primary antibody and incubate according to manufacturer's protocol.
17. Rinse 3 times in buffer.
18. Apply PolyTek Anti-Mouse Polymerized Alk-Phos (PAT) and incubate for 30 minutes at room temperature.
19. Rinse 2 times in buffer.
20. Rinse 1 time in Distilled/DI water.

Mix Permanent Red Concentrate with Permanent Red Buffer.

21. Combine 10µl of Permanent Red Concentrate with each 1ml of Permanent Red Buffer. Combined mixture may be used for up to two hours.
22. Apply Permanent Red Chromogen/Substrate mixture and incubate for a second 5 minute period.
23. Rinse 1 time in Distilled/DI water.
24. Apply mixture a second time and incubate for another 5 minutes.
25. Rinse slide using DI/Distilled water.
26. If desired, repeat application for a third time.
27. Apply Hematoxylin for Automation (HMM) and incubate for 30-60 seconds.
28. Rinse 3 times in distilled water.
29. Apply Bluing Reagent (BRT) and incubate for 5-10 seconds.
30. Rinse immediately in distilled or deionized water.
31. Quickly dehydrate in alcohol and clear in xylene or substitute.

Note: Alcohol and Xylene can cause chromogen to leach from tissue over extended periods of time.

Storage: 2° C  8° C


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32. Coverslip using a permanent mounting media.

-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 times were too long.

Non-Specific Background Staining:

1. Rinsing between steps was inadequate.
2. Tissue was allowed to dry with reagents on.
3. Folds in tissue trapped reagents.
4. Antigen migrated in tissue.
5. Excessive tissue adhesive on slides.
6. Inadequate blocking with protein 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 buffer between steps, resulting in dilution of reagents.
4. Room temperature was excessively cool.
5. The primary antibody does not recognize an antigen that survives fixation and embedding in high enough amounts.
6. Excessive incubation with protein block (Super Block or normal serum).

No Staining:

1. Steps were inadvertently left out.
2. There is no antigen in the tissue.
3. The primary antibody is not of mouse or rat origin.
4. Chromogenic substrate has been replaced with another that is not intended for use with alkaline phosphatase.
5. One or more components of the kit have been inactivated.

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