ScyTek Laboratories

Instructions For Use PAR-IFU		
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P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 - Fax (435) 755-0015 - www.scytek.com

PolyTek, Anti-Rabbit Polymerized HRP

Description: PolyTek, Anti-Rabbit Polymerized HRP has been developed to provide the cleanest, most consistent staining available. The system is based on a polymerized peroxidase label that eliminates biotin from the equation, thereby eliminating a major cause of background staining. In addition, this product reduces the steps required for immunohistochemical staining by combining two steps from the traditional Biotin-Streptavidin system.

Availability:

Item #	Volume
PAR008	8 ml
PAR015	15 ml
PAR125	125 ml
PAR500	500 ml
PAR999	1000 ml

Recommended, But Not Included:

Item #	Description
CPL500	Citrate Plus
ADA500	Peroxide Block for Image
AAA500	Super Block
ACT500	DAB Chromogen/Substrate Kit (High Contrast)
HAQ500	Hematoxylin for Automation
BRT500	Bluing Reagent

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.

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11. Once Coplin Jar cools to room temperature remove slides, rinse several times in buffer and proceed with staining as usual.

12. Apply Peroxide Block for Image Analysis (ADA) and incubate slide for 10-15 minutes.

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 3 times in buffer.

16. Apply rabbit primary antibody and incubate according to manufacturer's protocol.

17. Rinse 3 times in buffer.

18. Apply PolyTek, Anti-Rabbit Polymerized HRP (PAR) and incubate for 30 minutes at room temperature.

19. Rinse 3 times in buffer.

WARNING: DAB is a suspected carcinogen. Handle with care and dispose of according to all regulations.

20. Add 4 drops (200ul) DAB Chromogen (ACB) to DAB Substrate High Contrast (ACU), mix by swirling and apply to tissue for 5 minutes.

21. Rinse 1 time in buffer.

22. Apply DAB Chromogen/Substrate mixture and incubate for a second 5 minute period.

23. Rinse 3 times in buffer.

24. Apply Hematoxylin for Automation (HAQ) and incubate for 1 minute.

25. Rinse 3 times in distilled water.

26. Apply Bluing Reagent (BRT) and incubate for 5 seconds.

27. Rinse immediately in distilled or deionized water.

28. Dehydrate slides and clear in xylene or xylene substitute.

29. Coverslip using a permanent mounting media.

-Troubleshooting Guide-

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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.

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. 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 rabbit origin.
4. Chromogenic substrate has been replaced with another that is not intended for use with peroxidase.
5. One or more components of the kit have been inactivated.

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