



## Instructions For Use PAR-IFU

Rev. Date: 12/30/05

**Revision: 1** 

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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: <u>Item #</u> <u>Volume</u>

PAR008 8 ml
PAR015 15 ml
PAR125 125 ml
PAR500 500 ml
PAR999 1000 ml

### Recommended, But Not Included:

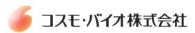
<u>ltem #</u>	<u>Description</u>
CPL500	Citrate Plus
ADA500	Peroxide Block for Image
AAA500	Super Block
ACT500	DAB Chromogen/Substrate Kit (High Contrast)
HAQ500	Hematoxylin for Automation

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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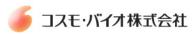
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- 11. Once Coplin Jar cools to room temperature remove slides, rinse several times in buffer and proceed with staining as usual.
- 12. Apply Peroxide Block for Image Analysis (ADA) and incubate slide for 10-15 minutes.
- 13. Rinse 3 times in buffer.
- 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.
- Folds in tissue trapped reagents.
- Antigen migrated in tissue.
- 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.