

Instructions For Use

Revision: 2

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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

UltraTek HRP Anti-Polyvalent Lab Pack

Description:

The UltraTek staining kit provides unmatched sensitivity with incubation times of 10 minutes each for the Link Antibody and Enzyme Label. The bulk kits are ideal for high volume laboratories. Each Pack contains one bottle of Super Block (universal protein block), one bottle of Biotinylated Antibody (Polyvalent), and one bottle of Horseradish Peroxidase Labeled Streptavidin. These Lab-Packs provide an extremely economical alternative for automated staining systems and we encourage you to evaluate the addition of this in your current system.

Species of Origin:	Goat
Antigen Specificity:	Anti-Polyvalent (Mouse, Rat, Rabbit and Guinea Pig).
Preadsorbed Against:	Human
Enzyme Conjugate:	Horseradish Peroxidase
Chromogen Substrate:	None Provided
-	

Contains:

One container of Super Block. One container of Anti-polyvalent. One container of HRP.

- 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 FFPE tissue. Any Fresh or Frozen tissue. Cell smear or spin.

Ordering Information and Current Pricing at www.scytek.com

Availability:

<u>ltem #</u> UHP125 UHP500 UHP999

<u>Volume</u> 125ml each 500ml each 1000ml each

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.



ScyTek Laboratories, Inc. 205 South 600 West Logan, UT 84321 U.S.A.



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Recommended, But *Not Included*:

or	<u>Item #</u> PBE500 TBE500	<u>Description</u> Phosphate Buffered Saline + Tween 20 (10x) pH 7.4 Tris Buffered Saline + Tween 20 (10x) pH 7.5
	CPL500 ADA500 ACT500 HMM500 BRT500	Citrate Plus Peroxide Block for Image DAB Chromogen/Substrate Kit (High Contrast) Hematoxylin, Mayer's (Lillie's Modification) 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. (Not included)
- 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. Apply Peroxide Block for Image Analysis (ADA) and incubate slide for 10-15 minutes. (Not included)
- 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 primary antibody and incubate according to manufacturer's protocol.
- 17. Rinse 3 times in buffer.
- 18. Apply UltraTek Anti-Polyvalent (ABN) and incubate for 10 minutes at room temperature.
- 19. Rinse 3 times in buffer.
- 20. Apply UltraTek HRP (ABL) and incubate for 10 minutes at room temperature.
- 21. Rinse 3 times in buffer followed by 1 rinse in DI water.

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

- 22. Add 1 drop (40-50ul) DAB Chromogen (ACB) to each 1ml of DAB Substrate High Contrast (ACU), mix by swirling and apply to tissue for 5 minutes. (*Not included*)
- 23. Rinse 1 time in DI water.

Storage: 2° C

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- 24. Apply DAB Chromogen/Substrate mixture and incubate for a second 5 minute period.
- 25. Rinse 3 times in buffer.
- 26. Apply Hematoxylin, Mayer's (HMM) and incubate for 1 minute. (Not included)
- 27 Rinse 3 times in distilled water.
- 28. Apply Bluing Reagent (BRT) and incubate for 5-10 seconds. (Not included)
- 29. Rinse immediately in distilled or deionized water.
- 30. Dehydrate slides and clear in xylene or xylene substitute.
- 31. Coverslip using a permanent mounting media. (Not included)

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 peroxidase.
- 5. Tissue contains endogenous biotin.
- 6. Antigen migrated in tissue.
- 7. Excessive tissue adhesive on slides.
- 8. 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 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).

Storage: 2° C

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