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-Mouse (AEC) Staining System

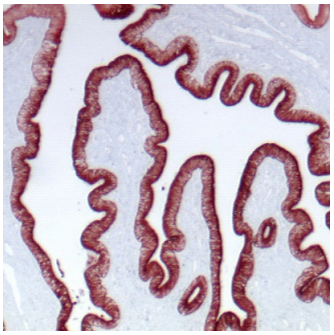
Description:

The UltraTek staining kit provides unmatched sensitivity with incubation times of 10 minutes each for the Link Antibody and Enzyme Label

Species of Origin:	Goat
Antigen Specificity:	Anti-Mouse
Preadsorbed Against:	Human
Enzyme Conjugate:	Peroxidase
Chromogen Substrate:	3-Amino-9-Ethylcarbazole (AEC)
Approximate Tests:	70 Slides

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

Kit Contents:

Item #	Volume
Peroxide Block	1 x 8 ml
Super Block	1 x 8 ml
UltraTek Anti-Mouse	1 x 8 ml
UltraTek HRP	1 x 8 ml
AEC Chromogen	1 x 3 ml
AEC Substrate Buffer	7 x 5 ml vials

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:

Storage: 2° C 8° C

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

CE IVD

EC REP
Emergo Europe
Prinsessegracht 20
2514 AP The Hague, The Netherlands

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1. Deparaffinize and rehydrate tissue section.
2. To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in hydrogen peroxide for 10-15 minutes.
3. Wash 2 times in buffer.
4. If required, incubate tissue in digestive enzyme.
5. Wash 4 times in buffer.
6. 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.
7. Wash 1 time in buffer.
8. Apply primary antibody and incubate according to manufacturer's protocol.
9. Wash 4 times in buffer.
10. Apply UltraTek Anti-Mouse (yellow cap), and incubate for 10 minutes at room temperature.
11. Wash 4 times in buffer.
12. Apply UltraTek HRP (red cap), and incubate for 10 minutes at room temperature.
13. Rinse 4 times in buffer.
14. Combine 2 drops of AEC Chromogen Concentrate to one vial of AEC Substrate Buffer. Combined mixture must be used immediately.
15. Dip slide one time in DI/Distilled water immediately prior to application of AEC.
16. Apply mixture to tissue section.
17. Incubate tissue section for 5 minutes.
18. Rinse slide using DI/Distilled water.
19. Apply mixture and incubate for another 5 minutes.
20. Rinse slide in two changes of DI/Distilled water.
21. If needed, repeat application, incubation, and rinse for a third 5 minute period.
22. Counterstain as desired.
23. Rinse slide in two changes of DI/Distilled water.
24. Coverslip in aqueous medium.

NOTE: Dehydrating in alcohol and clearing in xylene results in immediate loss of AEC from slide.

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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 Backgroung 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 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.
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.