



SPECIFICATION SHEET

## UltraVision Detection System Anti-Rabbit, HRP/AEC (Ready-To-Use)

### DESCRIPTION:

The reagents in this kit constitute a labeled streptavidin-biotin immunoenzymatic antigen detection system. This technique involves the sequential incubation of the specimen with an unconjugated primary antibody specific to the target antigen, a biotinylated secondary antibody which reacts with the primary antibody, enzyme-labeled streptavidin, and substrate-chromogen.

<b>SPECIES OF ORIGIN:</b>	Goat				
<b>ANTIGEN SPECIFICITY:</b>	Anti-Rabbit IgG (H+L)				
<b>PREADSORBED AGAINST:</b>	Human, Mouse, Rat				
<b>ENZYME CONJUGATE:</b>	Peroxidase				
<b>CHROMOGEN/SUBSTRATE:</b>	3-amino-9-ethylcarbazole (AEC)				
<b>AVAILABILITY:</b>	<table><tr><td><u>Catalog #</u></td><td><u>Slide Volume</u></td></tr><tr><td>TR-015-HA</td><td>150-300 slides</td></tr></table>	<u>Catalog #</u>	<u>Slide Volume</u>	TR-015-HA	150-300 slides
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TR-015-HA	150-300 slides				

### STAINING PROTOCOL (kit components in bold):

1. Deparaffinize and rehydrate tissue section.
2. To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in **Hydrogen Peroxide Block** for 10-15 minutes.
3. Wash 2 times in buffer.
4. If required, incubate tissue in digestive enzyme (or appropriate pretreatment).
5. Wash 4 times in buffer.
6. (Optional) Apply **Ultra V Block** 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. Rinse (Optional).
8. Apply primary antibody and incubate according to manufacturer's protocol.

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9. Wash 4 times in buffer.
  10. Apply **Biotinylated Goat Anti-Rabbit** and incubate for 10 minutes at room temperature.
  11. Wash 4 times in buffer.
  12. Apply **Streptavidin Peroxidase** and incubate for 10 minutes at room temperature.
  13. Rinse 4 times in buffer.
  14. Add 20µl of **AEC Chromogen** to 1 ml of **AEC Substrate** and mix well. Apply mixture to tissue section within 20 minutes of mixing. Incubate tissue section for 5-20 minutes, depending on the desired stain intensity.  
**WARNING:** AEC is a suspected carcinogen. Handle with care and dispose of according to all regulations.
  15. Counterstain and coverslip.
- NOTE:** AEC produces a red end product that is soluble in alcohol and must be used with an aqueous counterstain and mounting media.
- STORAGE:** Store at 2-8°C.

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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 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:

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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. 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 (Ultra 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.

**LIMITATIONS:**

This product is available for research use only; not for diagnostic or therapeutic work. Lab Vision Corporation will not be held responsible for patent infringement or other violation that may occur with the use of this product.