



SPECIFICATION SHEET

UltraVision Detection System Anti-Rabbit, Alk-Phos/Fast Red (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.

SPECIES OF ORIGIN:	Goat				
ANTIGEN SPECIFICITY:	Anti-Rabbit IgG (H+L)				
PREADSORBED AGAINST:	Human, Mouse, Rat				
ENZYME CONJUGATE:	Alkaline Phosphatase				
CHROMOGEN/SUBSTRATE:	Fast Red/Naphthol Phosphate Substrate				
AVAILABILITY:	<table><tr><td><u>Catalog #</u></td><td><u>Slide Volume</u></td></tr><tr><td>TR-015-AF</td><td>150-300 slides</td></tr></table>	<u>Catalog #</u>	<u>Slide Volume</u>	TR-015-AF	150-300 slides
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TR-015-AF	150-300 slides				

STAINING PROTOCOL (kit components in bold):

1. Deparaffinize and rehydrate tissue section.
2. Wash 2 times in buffer.
3. If required, incubate tissue in digestive enzyme (or appropriate pretreatment).
4. Wash 4 times in buffer.
5. (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.
6. Rinse (Optional).
7. Apply primary antibody and incubate according to manufacturer's protocol.

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TR-015-AF
Rev 21211
Page 1 Of 2



8. Wash 4 times in buffer.
9. Apply **Biotinylated Goat Anti-Rabbit** and incubate for 10 minutes at room temperature.
10. Wash 4 times in buffer.
11. Apply **Streptavidin Alkaline Phosphatase** and incubate for 10 minutes at room temperature.
12. Rinse 4 times in buffer.
13. Add 1 **Fast Red tablet** to 5 ml of **Naphthol Phosphate Buffer** and shake until tablet is dissolved. Apply to tissue section and incubate for 10-20 minutes or until desired stain intensity is achieved.
WARNING: Handle with care and dispose of according to all regulations.
14. Counterstain and coverslip using an aqueous 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 alkaline phosphatase.
5. Tissue contains endogenous biotin.
6. Antigen migrated in tissue.
7. Excessive tissue adhesive on slides.
8. Inadequate blocking with protein block.

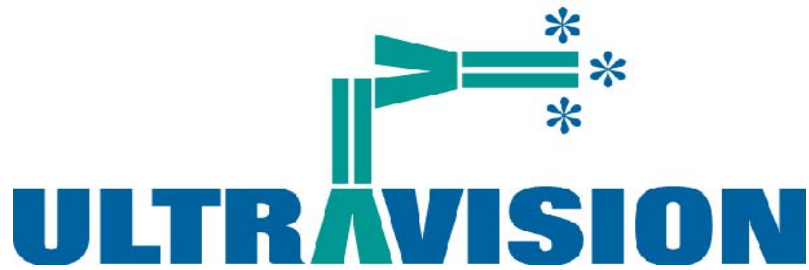
WEAK STAINING:

1. Primary antibody concentration was too low or incubation time was too short.

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TR-015-AF
Rev 21211
Page 2 of 2



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 alkaline phosphatase.
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. LabVision Corporation will not be held responsible for patent infringement or other violation that may occur with the use of this product.