

ScyTek  
Laboratories

Instructions For Use  
AFP600-IFU

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## UltraTek Alk-Phos (Anti-Polyvalent) Ready-To-Use (500 slide)

<b>Species of Origin:</b>	Goat
<b>Antigen Specificity:</b>	Anti-Polyvalent (Mouse, Rat, Rabbit and Guinea Pig.
<b>Preadsorbed Against:</b>	Human
<b>Enzyme Conjugate:</b>	Alkaline Phosphatase
<b>Chromogen Substrate:</b>	None Provided

### Procedure:

1. Deparaffinize and rehydrate tissue section.
2. Wash 2 times in buffer.
3. If required, incubate tissue in digestive enzyme.
4. Wash 4 times in buffer.
5. 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.
6. Wash 1 time in buffer.
7. Apply primary antibody and incubate according to manufacturer's protocol.
8. Wash 4 times in buffer.
9. Apply UltraTek Anti-Polyvalent (yellow cap), and incubate for 10 minutes at room temperature.
10. Wash 4 times in buffer.
11. Apply UltraTek Alk-Phos (red cap), and incubate for 10 minutes at room temperature.
12. Rinse 4 times in buffer.
13. Apply chromogen intended for use with Alkaline Phosphatase and incubate as desired.
14. Counterstain and coverslip using a aqueous mounting media.

### 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.

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**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.
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).

**No Staining:**

1. Steps were inadvertently left out.
2. There is no antigen in the tissue.
3. The primary antibody is not of mouse, rat, rabbit or guinea pig origin.
4. Chromogenic substrate has been replaced with another that is not intended for use with alkaline phosphatase.