

## Streptavidin-Peroxidase (50X)

**Description:** ScyTek's Streptavidin-Peroxidase has been optimized to produce extremely sensitive immunohistochemical staining when diluted 1:50. At the recommended concentration, incubations of 10 minutes are advised for clean, high quality stains. For most procedures, commercially available primary antibodies can be diluted up to 50% further than with other systems.

**Uses/Limitations:** Immunohistochemistry.  
Do not use past expiration date.  
Do not use a diluent that contains Sodium Azide.

**Storage:** Store at 2-8°C.

### Procedure:

1. Prior to use, dilute the reagent by adding 1ml of Streptavidin-Peroxidase (50x) to 49ml of Enzyme Label Diluent (REF # ABI) and mix gently.
2. Deparaffinize and rehydrate tissue section.
3. Rinse in buffer.
4. If required, incubate tissue in digestive enzyme.
5. Rinse in buffer.
6. (Optional) Place slide in Super Block (REF # 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.
7. Rinse in buffer.
8. Apply primary antibody and incubate according to manufacturer's protocol.
9. Rinse in buffer.
10. Place slide in link antibody, and incubate according to protocol.
11. Rinse in buffer.
12. Place slide in Streptavidin-Peroxidase, and incubate for 10 minutes at room temperature.
13. Rinse in buffer.
14. Place slide in appropriate chromogenic substrate and incubate until desired reaction is achieved.
15. Counterstain and coverslip.

### 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 enzyme.
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. Reagent is reaching the end of its useful life.
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 relevant antigen in the tissue.
3. The primary antibody does not match the biotinylated link antibody.
4. Chromogenic substrate does not match enzyme label.
5. One or more components have been inactivated.