AviBond Basic HRP Anti-Polyvalent (DAB) Ready-To-Use (70 slides)

Description

Species of Origin:	Goat
Antigen Specificity:	Anti-Mouse, Rat, Rabbit, Guinea Pig
Preadsorbed Against:	Human
Enzyme Conjugate:	Peroxidase
Chromogen Substrate:	Diaminobenzidine (DAB)

Contents

Product	Volume	Storage
IncrediBlock Basic	8 ml	2-8°C
PeroxiBlock Basic	8 ml	2-8°C
AvidBond Basic Anti-Polyvalent	8 ml	2-8°C
AviBond Basic HRP	8 ml	2-8°C
DAB Clarity Basic Chromogen	3 ml	2-8°C
DAB Clarity Basic Substrate	5 ml x 7 vials	2-8°C

Uses/Limitations

Not to be taken internally.
Do not use if reagent becomes cloudy.
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile.

Precautions

Avoid contact with skin and eyes.
Harmful if swallowed.
Follow all Federal, State, and local regulations regarding disposal.

Procedure

1. Deparaffinize and rehydrate tissue section.
2. To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in hydrogen peroxide for 5 minutes.
3. Wash 2 times in buffer.
4. If required, perform antigen retrieval following manufacturer's recommendations.
5. Wash 4 times in buffer.
6. Apply IncrediBlock Basic (blue cap), and incubate for 5-10 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 AviBond Basic Biotinylated Anti-polyvalent (yellow cap), and incubate for 30 minutes at room temperature.
11. Wash 4 times in buffer.
12. Apply AviBond Basic HRP (red cap), and incubate for 30 minutes at room temperature.
13. Rinse 4 times in buffer.
14. Add 4 drops (200 µl) DAB Clarity Basic Chromogen to DAB Clarity Basic Substrate, mix by swirling and apply to tissue. Incubate for 5-15 minutes, depending on the desired stain intensity.

    WARNING: DAB is a suspected carcinogen. Handle with care and dispose of according to all regulations.

15. Counterstain and coverslip

Troubleshooting

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.
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 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, rat, rabbit or guinea pig origin.
4. Chromogenic substrate has been replaced with another that is not intended for use with peroxidase.