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# IMUNOHISTOCHEMISTRY(PARAFFIN)(IHC-P)

**Important:** Please refer to the APPLICATIONS section on the front page of product datasheet or product webpage to determine whether a product is validated and approved for use on paraffin-embedded (IHC-P) tissue sections.

**Note:** Please see product webpage for appropriate antibody dilution, diluent and unmasking solution.

## Solutions and Reagents

1) Acetone
2) Ethanol, anhydrous denatured, histological grade (100% and 95%).
3) Deionized water ($dH_2O$).
4) Hematotoxylin
5) Xylene
6) 10X TBS
7) Wash Buffer TBST
8) Antibody Diluent
9) 10 mM Sodium Citrate Buffer
10) 3% Hydrogen Peroxide
11) Blocking Solution
12) ABC Reagent
13) Diaminobenzidine (DAB) Substrate
14) Mounting Medium

## Blocking step

1) Incubate sections with 3-10% normal serum from the same species as the secondary antibody, for 30 minutes to block non-specific binding of immunoglobulin.
2) Remove blocking solution.

## Primary antibody incubation

All steps should be performed in a moist environment.

1) Dilute the primary antibody in blocking solution. If no dilution is suggested, begin testing at 1:10, 1:100 and 1:200.

2) Incubation overnight at 4°C.
3) Remove antibody solution. Wash 3 x 5 minutes in PBS, pH 7.4.
4) If the primary antibody is HRP-conjugated, proceed to Color Development.

## Secondary antibody incubation

1) Dilute the biotin-conjugated secondary antibody in blocking solution according to the recommended dilution. Incubate 30-60 minutes at RT.
2) Removed secondary antibody solution. Wash slides 3 x 5 minutes in wash buffer.

## Development

1) Add streptavidin-horseradish peroxidase reagent to each section and incubate for 30 minutes at RT.
2) Remove ABC reagent. Wash sections 3x in wash buffer for 5 minutes each.
3) Apply the DAB solution to cover the sections completely in a moist environment. Incubate for 5-15 minutes at RT. Alternatively, observe the slide under a microscope to determine optimal color intensity of the insoluble precipitate.
4) As soon as color develops, stop the reaction by gently flushing with $dH_2O$.
5) Counterstain tissue as desired with Hematoxylin & Eosin to define antigen proximity to normally expected structures.
6) Wash the slides with $dH_2O$.

## Dehydration and coverslip application

1) Dehydrate the sample for storage using a series of methanol or ethanol graded concentrations: 50% (2 x 5 min), 75% (2 x 5 min), and finally 100% (2 x 5 min).
2) Repeat in xylene, incubating sections two times for 10 seconds each.
3) Allow the slide to air dry.
4) Mount coverslips using appropriate mounting media.