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UltraPolymer
IHC Staining Protocol

For use with ***UltraPolymer*** secondary antibody-HRP polymers. UltraPolymer reagents are optimized for use with FFPE tissue sections.

Protocol

1) Deparaffinize slides and perform antigen retrieval (download from the web site either Antigen Retrieval – Citrate Buffer or Antigen Retrieval – EDTA Buffer protocols as appropriate for use with your particular primary antibodies, see manufacturer's web sites for recommendations)
2) After flushing slides with running tap water for 5 min, put slides in hydrogen peroxide (3% in distilled water) for 10 min.
3) Rinse slides in distilled water by dipping a few times.
4) Wash slides in wash buffer for 5 min.
5) Dry slides around tissue sample and draw a ring with hydrophobic pen.
6) Block section with blocking buffer (see notes) for 10 minutes.
7) Prepare diluted primary antibody by diluting concentrate with antibody diluent (see notes). Aspirate blocking solution from the slide and then add 150 to 200ul per section and incubate for 1 hour at room temperature in a humidified chamber.
8) Wash slide with wash buffer, 3 changes for 5 min each.
9) Add 150 to 200ul UltraPolymer antibody per section and incubate for 1 hour at room temperature in a humidified chamber.
10) Wash slide with wash buffer, 3 changes for 5 min each.
11) Make DAB according to manufacturer's instructions and add directly to the slide. Let stain for 5-10 min.
12) Wash slides with wash buffer, 3 changes for 5 min each.
13) Rinse slides with tap water.
14) Dip slides in Hematoxylin for 30 sec to 1 min.
15) Rinse slides with tap water for 2 min until tissue samples looks blue.
16) Dehydrate tissue sample as follows:

95% Reagent alcohol	2 min
95% Reagent alcohol	2 min
100% Reagent alcohol	2 min
100% Reagent alcohol	2 min
Xylene	5 min
Xylene	5 min

17) Drop 3 drops of mounting medium directly onto slide and carefully place coverslip.

Notes

1. Citrate antigen retrieval buffer (10mM citrate, 0.05% Tween 20, pH 6.0) may be prepared from solid trisodium citrate dihydrate and stored as a concentrated 10X solution (100mM citrate, 0.5% Tween 20) in dH_2O, pH 6.0.
Dissolve 29.4g of trisodium citrate dihydrate in 950ml distilled water then bring pH to 6.0 using 1N NaOH. Add 5ml Tween 20 and mix well before bringing final volume to 1000ml

with distilled water. This solution can be stored for 3 months at room temperature or longer if stored at 4°C. From the concentrated 10X solution, 1X working solution can then be prepared as needed by further dilution of the 10X stock in dH_2O. Buffer pH = 6.0 should be confirmed prior to each use, as pH levels may change during storage. Download the Antigen Retrieval - Citrate Buffer protocol from the web site for antigen retrieval instructions.

2. EDTA antigen retrieval buffer (1mM EDTA, 0.05% Tween 20, pH 8.0) may be made from solid EDTA disodium salt dihydrate and stored as a concentrated 10X solution (10mM EDTA, 0.5% Tween 20) in dH_2O, pH 8.0.
   Dissolve 3.7g of disodium EDTA dihydrate in 950ml distilled water then bring pH to 8.0 using 1N NaOH. Add 5ml Tween 20 and mix well before bringing final volume to 1000ml with distilled water. This solution can be stored for 3 months at room temperature or longer if stored at 4°C. From the concentrated 10X solution, 1X working solution can then be prepared as needed by further dilution of the 10X stock in dH_2O. Buffer pH = 8.0 should be confirmed prior to each use, as pH levels may change during storage. Download the Antigen Retrieval - EDTA Buffer protocol from the web site for antigen retrieval instructions.

3. Antigen retrieval methods have been successfully validated for this reagent using pressure cookers and steamers. Microwave antigen retrieval has not been tested. If a pressure cooker or steamer is not available, a 100°C boiling water bath may be used, but results may vary. Do not immerse pyrex container in boiling water bath.

4. Wash Buffer: PBS/0.2% Tween 20 pH 7.2. Wash buffer should be freshly prepared and stored for no longer than a week to avoid contamination.

5. Blocking Buffer: PBS/3% normal animal serum/0.1% TritonX. Use serum from the same species from which the secondary antibody is derived. Blocking buffer should be freshly prepared.

6. Antibody Diluent: PBS/1% BSA/0.2% Tween 20. Antibody diluent should be freshly prepared.

Troubleshooting

Issue	Possible Cause(s)	Solution
No antigen signal	Tissue is negative for antigen	Include known positive control tissue in experimental design
	Imaging settings are not optimal	Adjust settings using positive control tissue
	Antibody did not bind	Always use freshly diluted antibody cocktails
High background	Blocking incomplete	Always use freshly prepared blocking buffer and IgG-free BSA
Tissue damaged	Antigen retrieval pH < 6.0	Check pH of antigen retrieval solution
	Antigen retrieval time > 30 min	Incubate in antigen retrieval buffer no longer than 30 min total
	Tissue poorly affixed to slide	Use positively charged glass slides (e.g. SuperFrost Plus)
	Tissue damaged by handling	Gently wash and rinse slides. If using rotator, use low speed
		Do not allow slides to come in contact with each other
		Use caution applying coverslip and do not adjust during drying.

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Version 5.15.17