

IMMUNOCYTOCHEMISTRY PROTOCOL
BUFFERS FOR IMMUNOSTAINING APAAP AND ABC
BUFFERS FOR IMMUNOSTAINING ABC

Tris buffered saline (TBS):

100 ml Tris base 0.5 M pH 7.6

60.7 g Trizma base (Sigma T #1503) in 800 ml dd H₂O water. pH with 12 M HCL and adjust volume to 1000 ml with water

100 ml 1.5 M Sodium chloride (BDH Merck #10241)

87.66 g NaCl made up to 1000 ml in ddH₂O.

800 ml distilled water

Substrate for alkaline phosphatase:

20 mg Naphthol AS-MX phosphate (Sigma N #4875)

2 ml Dimethylformamide (BDH #28425)

Mix together in a glass container

then add:

98 ml Tris buffer 0.1 M pH 8.2

12.11 g Trizma base in 800 ml .pH with 12 M HCL

Make up to 1000 ml

100 µl 1M Levamisole (Sigma L #9756)

0.1205 g Levamisole made up to 500 µl and stored in aliquots at - 20 °C

This **prepared substrate** can be stored at 4 °C up to 2 weeks before use.

Just before use add 1 mg/ml Fast red (Sigma F #2768) to substrate and mix to dissolve.

TO USE: Filter through a 0.2 µl syringe filter onto the tissue.

ABC Substrates & Chromagens: (Not applicable for APAAP)

HRP (horse radish peroxidase): use *Peroxidase Stabilizing Buffer* #D2100S (DAKO Diagnostics Can) to dilute the peroxidase complex.

DAB: use DAB substrate kit #D1300A & B (DAKO Diagnostics Can)

Just before use mix equal parts A & B together.

AEC: use AEC 1 step chromagen D1002S (DAKO Diagnostics Can)

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