IHC PROCEDURE

Solutions and reagents:

Neutral-buffered Formalin, 10% (NBF), 1 liter

(Commercially available pre-prepared from many laboratory reagent suppliers).

Double -distilled H2O	900 ml
Di-sodium hydrogen phosphate, anhydrous (Na_2HPO_4)	6.5 grams
Sodium di-hydrogen phosphate, monohydrate (NaH_2PO_4 . H_2O)	4.0 grams
Formaldehyde, 37% solution	100 ml

The pH should be about 7.0. Adjust if necessary with 1M NaOH or 1M HCl. Store at 4°C.

Final concentrations:

Formaldehyde	3.7%
Na_2HPO_4	46 mM
NaH_2PO_4. H_2O	29 mM

(Note: Formaldehyde, a gas highly soluble in water, is typically sold as a saturated aqueous solution of 37% by mass. This is also referred to as “100% formalin”. 10% neutral-buffered formalin is the 10-fold dilution of this solution, giving a final concentration of formaldehyde of 3.7%).

For recipes for other fixation buffers we recommend our more comprehensive IHC fixation guide.

Sodium Citrate Buffer pH 6.0, 1 liter

(Also available from Abcam in a 10X preparation: ab64214).

Tri-sodium citrate (di-hydrate)	2.9 g
Double distilled water	1000 ml

Mix to dissolve sodium citrate and adjust pH to 6.0 with 1M HCl.
Add 0.5 ml Tween 20. Store at room temperature or at 4°C if storing for longer than 3 months.

Final concentrations:

Sodium citrate	10 mM
Tween 20	0.05%

For recipes and methods for other antigen retreival buffers we recommend our more comprehensive IHC-P (immunohistochemistry-paraffin) guide.

TBST (Tris-Buffered Saline, 0.05% Tween20), 1 liter

(Also available from Abcam in a 20X preparation: ab64204).

10X TBS	100 ml
Double-distilled water	900 ml
Tween 20	0.5 ml

(Note: For 10X TBS recipe, see Buffers and stock solutions page).

Blocking buffer

5% serum, milk or BSA (bovine serum albumin) in TBST

(Note: Blocking buffer reduces the background signal produced by non-specific interaction of primary and secondary antibodies with proteins in the tissue section. Serum is required in the blocking solution to block immunoglobulin Fc receptors present on cells in the section. The serum should be of the same species as the secondary antibody).

Procedure:

1. Tissue Preparation:

Fix tissue in 10% neutral buffered formalin for at least 24 hours. Embed in paraffin wax according to embedding machine manufactures instructions. Formalin has a very slow diffusion coefficient so the tissue needs to be no more than 1 cm thick.

For recipes for other fixation buffers and methods we recommend to view our more detailed IHC fixation guide.

2. Tissue Sectioning:

Prepare 4 - 12 µm sections on the microtome and place on clean, positively - charged microscope slides. Heat in tissue-drying oven for 45 minutes at 60°C.

3. Deparaffinization:

Wash slides 3 times for 5 minutes in xylene.

4. Rehydration:

4.1Wash slides 3 times for 3 minutes in 100% alcohol.

4.2 Wash slides 2 times for 3 minutes in 95% alcohol.

4.3 Wash slides 2 times for 3 minutes in 80% alcohol.

4.4 Rinse slides for 5 minutes in running distilled water.

5. Antigen retrieval:

There are several methods of antigen retrieval. The most common is heat-mediated retrieval in citrate buffer.

5.1 Heat slides in 10 mM sodium citrate buffer, pH 6.0 at 95 -100℃ for 20 minutes.

5.2 Remove from heat and let stand at room temperature in buffer for 20 minutes.

5.3 Rinse in TBST 1 minute.

There are other antigen retrieval procedures available and the type of retrieval and the incubation time for antigen retrieval may require some optimization. For some further details, view the antigen retrieval section in our more detailed IHC-P guide:

6. Immunostaining:

(Do not allow tissues to dry at any time during the staining procedure).

6.1 Add 100 µl per slide of blocking solution. Incubate 20 to 30 minutes at room temperature.

6.2 Drain the blocking solution from slides. Apply 100 µl per slide of diluted primary antibody at recommended concentration. Incubate 45 minutes at room temperature or overnight at 4°C.

6.3 Wash slides in 1X TBST 4 times for 5 minutes

6.4 Apply a 100 µl per slide of diluted conjugated secondary antibody. Incubate for 30 minutes at room temperature.

6.5 Wash slides in 1X TBST 4 times for 5 minutes.

6.6 Apply color development (i.e. enzyme substrate) 30 minutes, or follow manufacturer's instructions.

6.7 Wash slides in 1X TBST 4 times for 5 minutes

6.8 6.8 Wash slides in distilled water for 1 minute.

7. Dehydrate and mount slides:

This method should only be used if the chromogen substrate is alcohol insoluble.

7.1 Wash slides in 2 changes of 80% alcohol, 1 minute each.

7.2 Wash slides in 2 changes of 95% alcohol, 1 minute each.

7.3 Wash slides in 3 changes of 100% alcohol, 1 minute each.

7.4 Wash slides in 3 changes of xylene 1 minute each.

7.5 Apply coverslip

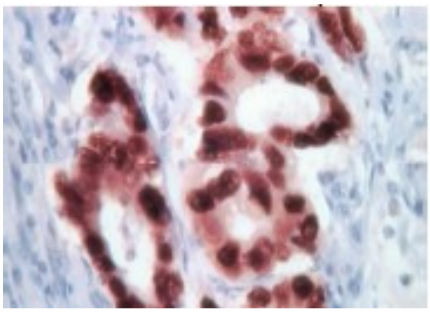
Useful links:

View our list of ready prepared positive control slides

View our DAB color detection kits

Example:

Anti-rabbit HRP DAB detection kit ab64261



Human colon carcinoma fixed in 10% NBF for 24 hrs and stained with anti p53 antibody using Anti Rabbit HRP/DAB Detection Kit (ab64261).