

Immunohistochemical staining of TNC in FFPE tissue

Sigma Monoclonal Mouse Anti-Human TNC (Clone BC-24) Code T2551

- Positive control: tonsil, asthma bronchial biopsy (post allergen preferred)
- Negative control: as above with primary antibody omitted
- Antibody storage: -20C
- Antigen location: cytoplasmic expression in several cell types, extracellular matrix, basement membrane

1 day protocol

Special precautions: Do not let sections dry out at any time during this procedure!

Take slides to water:

Xylene	5 minutes
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Pre/post-Xylene IMS	5 minutes
100% IMS	5 minutes
90% IMS	5 minutes
75% IMS	3 minutes
50% IMS	1 minute
Running tap water	1 minute

Place slides in 1xTBS to equilibrate for 5 minute.

ImmEdge pen:

Draw a circle around the section using an ImmEdge Pen (Vector). Return to 1xTBS as you go along to ensure slides never dry out.

Antigen retrieval:

Pepsin Antigen retrieval (Sigma R2283, store 2-8C) – add 2-4 drops of Pepsin Reagent to cover the tissue section. Incubate at 37C for 10 minutes. Discard Pepsin reagent and wash 3xTBS 5 minutes

Endogenous peroxidase block:

Using Bloxall from the Vector ImmPRESS Excel Anti-Mouse Kit (cat no MP-7602), place one drop per section (or more if necessary to ensure coverage of the section) and incubate for 10 minutes. Wash in 1xTBS for 5 minutes.

Serum block:

Incubate sections in 2.5% normal horse serum (from Vector ImmPRESS Excel Anti-Mouse Kit) for 20 minutes. Tip off (except for negative section).

Primary antibody:

Sigma TNC (T2551) should be made up in 2.5% normal horse serum diluted in 1xTBS. Dilution of TNC is 1 in 4000. The negative section should remain in the normal horse serum from the kit. Incubate with TNC for 30 minutes at room temperature. Wash in 1xTBS for 5 minutes.

Amplifier antibody:

Incubate for 15 minutes in the amplifier antibody from the Vector ImmPRESS Excel Anti-Mouse Kit. Wash for 5 minutes in 1xTBS.

Secondary antibody:

Incubate for 30 minutes in ImmPRESS Excel reagent from the Vector ImmPRESS Excel Anti-Mouse Kit. Wash in 1xTBS for 2 x 5 minutes.

Chromogen:

Combine equal volumes of ImmPACT DAB EqV reagent 1 with reagent 2 (from the Vector ImmPRESS Excel Anti-Mouse Kit). Mix well. Incubate in this working solution for 30 seconds. Wash in running tap water for > 1 minute. Place in distilled water.

Counterstain:

Counterstain by dropping on enough Vector QS haematoxylin to cover the section. Wash off and blue in running tap water for > 1 minute.

Dehydrate, clear and mount:

50% IMS	3 minutes
75% IMS	5 minutes
90% IMS	5 minutes
100% IMS	5 minutes
Pre/post xylene IMS	5 minutes
Xylene	>5 minutes

Mount using DPX and leave to dry in the fume hood for at least 30 minutes before viewing.

Results

TNC positive	brown
Nuclei	blue

