

TROMA I Immunohistochemistry Protocol

DAY 1

Deparaffinize and Rehydrate

- Put into citrisolv for 2 hours
- 100% alcohol for 5 min -----2X
- 95% alcohol for 5 mins -----2X
- 70% alcohol for 5 mins -----2X
- dH2O for 15 mins

Block endogenous peroxidase

- Incubate in a solution mixed as written in the recipe below for 20 mins
- 42.60 mls of dH2O
- 2.50 mls of MeOH
- 4.16 mls of H2O2

Rinse in 1X PBS for 2 mins-----4X

Antigen Retrieval

- Incubate in a solution of (100 ul of Protinase K + 50mls of 1X PBS) for 20mins

Rinse in 1XPBS for 2mins-----4X

Incubate over night in a Primary Antibody solution of (9 parts PBS:1DMSO) + 1:100 TROMA I

DAY 2

Rinse in 1XPBS for 2mins-----4X

Incubate in Secondary Antibody at RT for 1.5 hrs in BA anti-rat (1:200)

Rinse in 1XPBS for 2mins-----4X

Incubate in Secondary Antibody at RT for 1.5 hrs in HRP-streptavidin (1:200) in a solution of 9:1 1XPBS:DMSO

Rinse in 1XPBS for 2mins-----4X

DAB Staining

- 20mg of DAB 2 tablets from -20 freezer
- 30mg of NiCl Nickle chloride
- make to 50mls with 1XPBS
- filter solution
- 96ul of H2O2

Observe for color development and rinse once you think color has developed. Approx. 10-15mins

Rinse in 1XPBS for 2mins-----4X