

## NB100-142 Protocol

### Immunohistochemistry-Paraffin Protocol for Mre11 Antibody (NB100-142)

Standard protocol for IHC validation

Tissue Preparation: Formalin fixation and embedding in paraffin wax

The tissue needs to be fixed overnight in formalin at room temperature (approx. 16 hours).

There needs to be at least a 10:1 ratio of formalin volume to volume of the tissue.

The tissue can be no more than 4-5 mm thick. If it is thicker than this, it will need to be bivalved (bisected so as to make two similar separate parts) before being added to the formalin due to the diffusion coefficient of formalin.

The working area of the tissue cassettes is roughly 2.5 cm by 3.0 cm. So the cut tissue sample can be no larger than this.

Formalin refers to 10% neutral buffered formalin.

After formalin fixation overnight tissues can be processed according to the following protocol:

Tissue Sectioning: Make 4-um sections and place on pre-cleaned and charged microscope slides.

Heat in a tissue-drying oven for 45 minutes at 60 degrees C.

Deparaffinization: Wash dry slides in 3 changes of xylene for 5 minutes each @ RT

Rehydration: Wash slides in 3 changes of 100% alcohol for 3 minutes each @ RT

Wash slides in 2 changes of 95% alcohol for 3 minutes each @ RT

Wash slides in 1 change of 80% alcohol for 3 minutes @ RT

Rinse slides in gentle running distilled water for 5 minutes @ RT

Antigen retrieval: Steam slides in 0.01 M sodium citrate buffer, pH 6.0 at 99-100 degrees C - 20 minutes

Remove from heat and let stand at room temperature in buffer - 20 minutes

Rinse in 1X TBS with Tween (TBST) at 1 minute @ RT

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

Apply a universal protein block for 20 minutes @ RT

Drain protein block from slides, apply diluted primary antibody for 45 minutes @ RT

Rinse slides in 1X TBST - 1 minute @ RT

Apply a biotinylated anti-rabbit IgG (H+L) secondary for 30 minutes @ RT

Rinse slides in 1X TBST - 1 minute @ RT

Apply alkaline phosphatase streptavidin for 30 minutes @ RT

Rinse slides in 1X TBST - 1 minute @ RT

Apply alkaline phosphatase chromogen substrate for 30 minutes @ RT

Wash slides in distilled water for 1 minute @ RT

Dehydrate: (This method should only be used if the chromogen substrate is alcohol insoluble (e.g. Vector Red, DAB))

Wash slides in 2 changes of 80% alcohol for 1 minute each @ RT

Wash slides in 2 changes of 95% alcohol for 1 minute each @ RT

Wash slides in 3 changes of 100% alcohol for 1 minute each @ RT

Wash slides in 3 changes of xylene for 1 minute each @ RT

Apply coverslip