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NB110-89719 Protocol

Immunohistochemistry Protocol specific for Ki67 Antibody (NB110-89719)

[[URL:https://www.novusbio.com/products/ki67-mki67-antibody_nb110-89719]][[Caption:Ki67/MKI67 Antibody]]

Antigen Unmasking

Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0 then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.

Wash sections in dH₂O three times for 5 minutes each.

Wash section in wash buffer for 5 minutes.

Block each section with 100-400 ul blocking solution (1X PBST, 5% goat serum) for 1 hour at room temperature.

Remove blocking solution and add 100-400 ul primary antibody diluted in 1X PBST, 5% goat serum to each section.

Incubate overnight at 4C.

Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.

Add 100-400 ul biotinylated secondary antibody, diluted in 1X PBST, 5% goat serum. Incubate 30 minutes at room temperature.

Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.

Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.

Wash sections three times in wash buffer for 5 minutes each.

Add 100-400 ul DAB substrate to each section and monitor staining closely.

As soon as the sections develop, immerse slides in dH₂O.

Wash sections in dH₂O two times for 5 minutes each.

Mount coverslips.