

NB600-246 Protocol

Immunohistochemistry-Frozen protocol for FoxP3 Antibody (NB600-246)

FoxP3 Antibody: https://www.novusbio.com/products/foxp3-antibody_nb600-246

IHC Protocol for NB600-246

Control Tissues: Thymus from healthy (C57BL6/6) and Influenza infected mice.

-Cut 5 um sections in a cryostat and fix them in cold acetone for 10 mins.

-Dry the slides completely at room temp for 30 mins.

-Permeabilize tissues by immersing them in 0.1% saponin, 3 times (5 mins each time).

-Block unspecific binding to the slides using 5% normal donkey serum, 5% normal rat serum and Fc block (1:500).

Everything has to be dissolved in 0.1% saponin.

-Incubate slides in a wet chamber at RT for 30 mins.

-Dip off the blocking solution from the slides and add streptavidin (0.1mg/ml) for 15 mins at RT.

-Wash one time with 0.1% saponin.

-Add similar volume of biotin (0.1) and incubate 15 min at RT.

-Wash one time with 0.1% saponin.

-Add NB600-246 (1:150) ON at RT.

-Next day: Wash slides with 0.1% saponin

-Add biotin-donkey anti-rabbit secondary (NB120-6801) for 3 hrs at RT.

- Wash slides with 0.1% saponin

-Add fluorescently labeled streptavidin (Molecular probes) in PBS for 1 hr at RT.

-Wash 3x with PBS.

-Mount with prolong gold antifade (Molecular probes)