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## NB600-245 Protocol

# Protocol specific for FOXP3 Antibody (NB600-245)

Protocol for Foxp3 staining.

- Cut 5 mm sections in a cryostat and fix them in cold acetone for 10 minutes
- Dry the slides completely at room temperature (30 minutes)
- Permeabilize tissues by immersing them in 0.1% saponin, 3 times (5 minutes 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 minutes
-Dip off the blocking solution from slides and add streptavidin (0.1 mg/ml) for 15 minutes at RT
-Wash one time with 0.1% saponin
-Add a similar volume of biotin (0.1 mg/ml) and incubate 15 min at RT
- Wash again with 0.1% saponin
- Add rabbit anti-Foxp3 (1:150) and biotin-rat anti-mouse CD4 (1:100) and incubate ON at RT
- Next day: Wash slides with PBS and add SA-594 or SA-488 (dissolved in PBS) to detect CD4 T cells (Dilution 1:500 from molecular probes). Incubate 1 h at RT
-Wash one time with 0.1% saponin and add biotin-donkey anti rabbit (Fab, Jackson Immunoresearch). Secondary antibody is diluted in PBS 1:500 and incubated by 3 hrs.
- Wash with saponin again and add SA-488 or SA-594 (1:500 in PBS). Incubate 1 h at RT
- Wash with PBS 3 times (10 minutes each wash)
- Mount slides with prolong gold antifade (Molecular probes)

Control: Incubated only with CD4 and no Foxp3
Tissue: thymus from an Influenza-infected mouse or thymus from healthy mice (C57BL/6)