Immunofluorescence protocol for frozen mice & rat brain sections

Thaw	Thaw sections for 5 min at room temperature	5
Fixation	• Mark section surrounding with special "border" pen or nail polish • Cover section either with 4% PFA (0.1M PB) for 6min at room temperature	10
Wash	• Briefly rinse in PB • Wash section 3x5 min in 0.1M PB on shaker platform	15

Block	• BSA solution for 60 min, at room temperature
	  ○ 3% Donkey serum, 1% BSA, 0.3% Tx, 0.1M PB
	  ○ Cover sections after adding serum and add wet paper to storage box to prevent dry out
		60

Primary antibody	• Primary Anti-B in BSA solution, for 60min at room temperature
	  ▪ Cover 1st half of the sections with primary antibody
	  ▪ Cover 2nd half of section with BSA solution -> control sections
	  ○ Adding wet paper to the storage box and close with lid
		60

Wash	• Rinse & Wash 3x5min in 0.1M PB on shaker platform	8

Secondary antibody	• Secondary Anti-B in BSA solution for 60 min at room temperature
	  ○ Produce decided concentration: 1:1000
	  ▪ Add 2nd antibody to solution
	  ▪ Cover all sections with solution
	  ▪ Add wet paper and close lid in the dark
		60

Wash	• Rinse & Wash 3x5min in 0.1 M PB on shaker platform in the dark	10
Mount	• Dip slides in MilliQ water to prevent crystal formation • Store slides in dark until dehydrated • Mount with entellan neu, coverslip and store in dark at +4C	120

Solutions:

- use ca. 300 µl per glass slide (if not divided into sections) for each step

- **0.1 M phosphate buffer** (2L, for one run per 25 glass slides)
  ○ 2 L MilliQ water
  ○ + 21.8 g Na2HPO4
  ○ + 6.4 g NaH2PO4
  ○ Mix well until dissolved, adjust pH to 7.4 and store at +4C
	- **4% PFA in 0.1 M PB**
	  ○ 10 ml 0.1 M PB
	  ○ + 400 mg Paraformaldehyde
	  ○ Heat to just under +58 C
	  ○ Add NaOH until clear
	  ○ Cool solution, adjust pH to 7.4
	  ○ Filter solution and store at +4C

- **BSA solution (Blocking & Incubation)**
  ○ 10 ml 0.1 PB
  ○ + 300 µl donkey serum (3%)
  ○ **+ 100 mg BSA (1%)**
  ○ **+ 30 µl Triton X 100 (0.3%)**
  ○ **Mix well and store at +4C for 2-3 days**	