

Immunofluorescence protocol for 4% PFA perfusion fixated mouse brain tissue, free-floating staining procedure

Day 1

Transfer	<ul style="list-style-type: none"> Transfer tissue from cryoprotectant to mesh inserts 	5
Wash	<ul style="list-style-type: none"> Wash sections 4x 5 min in 1x PBS (0.1M) <ul style="list-style-type: none"> Wash with 2 ml per well – all steps gentle shaking 	20
NaBH ₄	<ul style="list-style-type: none"> Incubate section in fresh 1% NaBH₄ in PBS for 20 min <ul style="list-style-type: none"> Incubations with 1.5 ml per well in provided 12 well plates 	20
Wash	<ul style="list-style-type: none"> Wash sections 4x 5 min in 1x PBS 	20
Blocking	<ul style="list-style-type: none"> Block sections for 60 min in 10% donkey serum (Sigma D9663) & 0.3% Triton X100 in PBS <ul style="list-style-type: none"> Prepare enough for blocking, primary and secondary antibody steps 	60
Wash	<ul style="list-style-type: none"> Wash sections 2x 5 min in PBS 	10
Primary Antibody	<ul style="list-style-type: none"> Incubate sections in 1% donkey serum & 0.3% Triton X100 in PBS with primary antibodies (double staining) overnight at +4C – ideally more than 16h 	O/N

Day 2

Wash	<ul style="list-style-type: none"> Wash 4x 5min in PBS 	20
Secondary antibody	<ul style="list-style-type: none"> Secondary Anti-B in 1% donkey serum & 0.3% Triton X100 in PBS for 1-2 h at room temperature protected from light 	120
Wash	<ul style="list-style-type: none"> Wash 4x5min in PBS 	20
Rinse	<ul style="list-style-type: none"> Rinse thoroughly in H₂O (MilliQ) 	5
Incubate (strengthen signal)	<ul style="list-style-type: none"> Incubate for 60 min in 1 mM CuSO₄ (in 50 mM ammonium acetate, pH 5.0, MilliQ) <ul style="list-style-type: none"> Ammonium acetate (sigma A7330) make up at 50mM at pH 5.0 with HCl Add CuSO₄-5H₂O (sigma C7631) to 1mM 	60
Rinse	<ul style="list-style-type: none"> Rinse thoroughly in H₂O (MilliQ) 	5
Wash	<ul style="list-style-type: none"> Wash 4x 5min in PBS 	20
Mount	<ul style="list-style-type: none"> Mount sections on polylysine coated slides in H₂O (MilliQ) Air dry (remove excess H₂O) and coverslip with Entellan New 	5 min per slide

Duration Day 1: 2h + 15 min; Day 2: 4h + 15 min