

PROTOCOL-AT-A-GLANCE

TISSUE SAMPLING & PREPARATION
(MOUSE BRAIN)

1. PERFUSION FIXATION

NaCl 9g/L + Heparin 10U/ml (not provided)	10mL	x 1	intracardiac perfusion according to standard protocol
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2 mL FIX-REAG (10X) + 18 mL FIX-BUFF 1X	20mL	x 1	intracardiac perfusion according to standard protocol
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2. POST-FIXATION

0,5 mL FIX-REAG (10X) + 4,5 mL FIX-BUFF 1X	5 mL	x 1	⌚ 3h	🌡️ 2-8°C
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WASH 1

200 µL Wash 1 (25X) + 4,8 mL dH2O	5mL	x 5	⌚ 30min	🌡️ 20-25°C
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CRYOPROTECTION

Wash 1 + 30% (w/v) sucrose	10 ml	x 2 (Slow agitation)	⌚ 24h	🌡️ 2-8°C
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FREEZING

🌡️ -80°C



3. SLICING & STORAGE

Slice organs, dry and store the slides in -80°C freezer until use.	🌡️ -80°C
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PROTOCOL-AT-A-GLANCE

TISSUE SECTION STAINING



1. DRYING SLIDES

⌚ 30min 🌡️ 20-25°C



WASH 1

40 µL Wash 1 (25X)
+ 960 µL dH₂O /slide

1mL

x 1

⌚ 10min 🌡️ 20-25°C



2. PERMEABILIZATION

PERM-SOLN diluted
1/2 (v/v) with Wash 1

0.5 mL

x 1

⌚ 10min 🌡️ 20-25°C
(in wet chamber)

WASH 1

40 µL Wash 1 (25X)
+ 960 µL dH₂O

1mL

x 3

⌚ 10min 🌡️ 20-25°C



3. STABILIZATION

STAB-REAG
+ STAB-BUFF

0.5 mL

x 1

⌚ 1hour 🌡️ 20-25°C



WASH 1

40 µL Wash 1 (25X)
+ 960 µL dH₂O

1mL

x 3

⌚ 10min 🌡️ 20-25°C



4. SATURATION

Ready to use

0.5 mL

x 1

⌚ 30min 🌡️ 20-25°C
(in wet chamber)

5. PRIMARY ANTIBODY

1 µL Primary antibody
+ 499 µL LAB-DILUT

0.5 mL

x 1

⌚ O/N 🌡️ 2-8°C
(in wet chamber)

WASH 2

40 µL Wash 2 (25X)
+ 960 µL dH₂O

1mL

x 3

⌚ 10min 🌡️ 20-25°C



6. SECONDARY ANTIBODY

Secondary antibody
not provided

0.5 mL

x 1

⌚ 1hour 🌡️ 20-25°C



WASH 2

40 µL Wash 2 (25X)
+ 960 µL dH₂O

1mL

x 3

⌚ 10min 🌡️ 20-25°C
(Protect from light)

WASH 3

40 µL Wash 3 (25X)
+ 960 µL dH₂O

1mL

x 3

⌚ 10min 🌡️ 20-25°C
(Protect from light)

7. MOUNTING & IMAGING

Mounting media
not provided