

## 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 dH <sub>2</sub> O	5mL	x 5	⌚ 30min	🌡️ 20-25°C
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**CRYOPROTECTION**

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

🌡️ -80°C
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**3. SLICING & STORAGE**

Slice organs, dry and store the slides in -80°C freezer until use.	🌡️ -80°C
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## TISSUE SECTION STAINING



	<b>1. DRYING SLIDES</b>					🕒 30min	🌡️ 20-25°C
	<b>WASH 1</b>	40 µL Wash 1 (25X) + 960 µL dH2O /slide	1mL	x 1	🕒 10min	🌡️ 20-25°C	
	<b>2. PERMEABILIZATION</b>	PERM-SOLN diluted 1/2 (v/v) with Wash 1	0.5 mL	x 1 (in wet chamber)	🕒 10min	🌡️ 20-25°C	
	<b>WASH 1</b>	40 µL Wash 1 (25X) + 960 µL dH2O	1mL	x 3	🕒 10min	🌡️ 20-25°C	
	<b>3. STABILIZATION</b>	STAB-REAG + STAB-BUFF	0.5 mL	x 1	🕒 1hour	🌡️ 20-25°C	
	<b>WASH 1</b>	40 µL Wash 1 (25X) + 960 µL dH2O	1mL	x 3	🕒 10min	🌡️ 20-25°C	
	<b>4. SATURATION</b>	Ready to use	0.5 mL	x 1 (in wet chamber)	🕒 30min	🌡️ 20-25°C	
	<b>5. PRIMARY ANTIBODY</b>	1 µL Primary antibody + 499 µL AB-DILUT	0.5 mL	x 1 (in wet chamber)	🕒 O/N	🌡️ 2-8°C	
	<b>WASH 2</b>	40 µL Wash 2 (25X) + 960 µL dH2O	1mL	x 3	🕒 10min	🌡️ 20-25°C	
	<b>6. SECONDARY ANTIBODY</b>	Secondary antibody not provided	0.5 mL	x 1	🕒 1hour	🌡️ 20-25°C	
	<b>WASH 2</b>	40 µL Wash 2 (25X) + 960 µL dH2O	1mL	x 3 (Protect from light)	🕒 10min	🌡️ 20-25°C	
	<b>WASH 3</b>	40 µL Wash 3 (25X) + 960 µL dH2O	1mL	x 3 (Protect from light)	🕒 10min	🌡️ 20-25°C	
	<b>7. MOUNTING &amp; IMAGING</b>	Mounting media not provided					