




































PROTOCOL-AT-A-GLANCE  
**WHOLE MOUNTS**


	<b>1. FIXATION</b>	100 $\mu$ L FIX-REAG (10X) + 900 $\mu$ L FIX-BUFF (1X)	1mL	x 1	 3h	 2-8°C
	<b>WASH 1</b>	200 $\mu$ L Wash 1 (25X) + 4.8 mL dH <sub>2</sub> O	5mL	x 5 (Agitation)	 30min	 2-8°C
	<b>2. PERMEABILIZATION</b>	Ready to use	1mL	x 1 (Slow agitation)	 1h	 20-25°C
	<b>WASH 1</b>	200 $\mu$ L Wash 1 (25X) + 4.8 mL dH <sub>2</sub> O	5mL	x 2 (Agitation)	 3min	 20-25°C
	<b>3. STABILIZATION</b>	STAB-REAG + STAB-BUFF	1mL	x 1 (Protect from light)	 1h	 20-25°C
	<b>WASH 1</b>	200 $\mu$ L Wash 1 (25X) + 4.8 mL dH <sub>2</sub> O	5mL	x 3 (Agitation)	 3min	 20-25°C
	<b>4. SATURATION</b>	Ready to use	1mL	x 1 (Slow agitation)	 1h	 20-25°C
	<b>5. PRIMARY ANTIBODY</b>	2 $\mu$ L Primary antibody + 998 $\mu$ L AB-DILUT	1mL	x 1 (Slow agitation)	 72h	 2-8°C
	<b>WASH 2</b>	200 $\mu$ L Wash 2 (25X) + 4.8 mL dH <sub>2</sub> O	5mL	x 5 (Agitation)	 30min	 20-25°C
	<b>6. SECONDARY ANTIBODY</b>	Secondary antibody not provided	1 mL	x 1 (Slow agitation)	 O/N	 2-8°C
	<b>WASH 2</b>	200 $\mu$ L Wash 2 (25X) + 4.8 mL dH <sub>2</sub> O	5mL	x 3 (Agitation - Protect from light)	 30min	 20-25°C
	<b>WASH 3</b>	200 $\mu$ L Wash 3 (25X) + 4.8 mL dH <sub>2</sub> O	5mL	x 3 (Agitation - Protect from light)	 30min	 20-25°C
	<b>7. MOUNTING &amp; IMAGING</b>	Mounting media not provided				