














PROTOCOL-AT-A-GLANCE

CELL CULTURE (24-well plate)



	WASH 1	Remove cell culture medium and wash	1 mL	x 1	🕒 1min	🌡️ 20-25°C
	1. FIXATION	2.5 µL FIX-REAG (200X) + 497.5 µL FIX-BUFF. 1X	0.5 mL	x 1	🕒 10min	🌡️ 20-25°C
	WASH 1	40 µL Wash 1 (25X) +960 µL dH2O	1 mL	x 3	🕒 3min	🌡️ 20-25°C
	2. PERMEABILIZATION	50µL PERM-SOLN (10X) + 450µL Wash 1	0.5 mL	x 1	🕒 10min (Slow agitation)	🌡️ 20-25°C
	WASH 1	40 µL Wash 1 (25X) +960 µL dH2O	1 mL	x 3	🕒 3min (agitation)	🌡️ 20-25°C
	3. STABILIZATION	STAB-REAG + STAB-BUFF	0.5 mL	x 1	🕒 10min	🌡️ 20-25°C
	WASH 1	40 µL Wash 1 (25X) +960 µL dH2O	1 mL	x 3	🕒 3min (agitation)	🌡️ 20-25°C
	4. SATURATION	Ready to use	0.5 mL	x 1	🕒 30min (Slow agitation)	🌡️ 20-25°C
	5. PRIMARY ANTIBODY	2 µL Primary antibody + 998 µL AB-DILUT	0.5 mL	x 1	🕒 0 / N (Slow agitation)	🌡️ 2-8°C
	WASH 2	40 µL Wash 2 (25X) + 960 µL dH2O	1 mL	x 3	🕒 10min (agitation)	🌡️ 20-25°C
	6. SECONDARY ANTIBODY	Secondary antibody not provided	0.5 mL	x1	🕒 1hour (Protect from light)	🌡️ 20-25°C
	WASH 2	40 µL Wash 2 (25X) + 960 µL dH2O	1 mL	x 3	🕒 3 min (Agitation - Protect from light)	🌡️ 20-25°C
	WASH 3	40 µL Wash 3 (25X) + 960 µL dH2O	1 mL	x 3	🕒 3min (Agitation - Protect from light)	🌡️ 20-25°C
	7. MOUNTING & IMAGING	Mounting media not provided				