proteintech®
Antibodies | ELISA kits | Proteins

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PRODUCT-SPECIFIC PROTOCOLS

IF (14682-1-AP)

Sample type	Fixation method	Blocking buffer	Antibody dilution buffer	Primary antibody dilution	Incubation time
U-251 cells	4% PFA in 1×PBS, 10 min at room temp	5% goat serum in 1×PBS	1% BSA in 1×PBS	1:400	1.5 h at room temp
	Secondary antibody		Secondary antibody dilution	Incubation time	Mounting media
	CoraLite®488-Conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) (SA00013-2)		-	1.5 h at room temp	Hydromount? mounting media with DAPI

PROTOCOL (more details available upon request):

1. Seed cells on sterile glass coverslips and gently wash the coverslip with 1X PBS.
2. Fix the samples with 4% PFA in 1×PBS, 10 min at room temp.
3. Rinse coverslip with 1XPBS 3 times for 5 min each.
4. Incubate the samples with 0.2% Triton X-100 made in 1XPBS for 5 min at room temperature.
5. Rinse the samples 3 times with 1XPBS for 3 min each.
6. Incubate the samples with Blocking buffer at room temperature for 1 h.
7. Aspirate Blocking buffer and incubate with primary antibody at room temperature for 2 hours or at 4°C for overnight.
8. Wash samples with 1XPBS 3 times for 5 min each.
9. Incubate with fluorochrome-labeled secondary antibody diluted in Antibody dilution buffer.
10. Wash coverslip with 1XPBS 3 times for 3 min each.
11. Mount and seal the sample, then visualize the target antigen using a fluorescent microscope.

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