NEUROMICS

Cripto1 Data Sheet

Catalog Number:	RA25091 & RA25091H (HRP Conjugated).	**Host:**	Rabbit
Product Type:	Affinity Purified	**Species Reactivity:**	Human, Mouse
Immunogen Sequence:	A synthetic peptide made to an internal portion of the human cripto protein sequence (between residues 100-150).	**Format:**	Liquid. Tris-glycine, 150mM NaCl and 0.05% Sodium Azide as a preservative. Concentration: 1.0 mg/ml.
Applications:	Immunofluorescence/Immunocytochemistry: 1:20-1:100 Flow Cytometry: 1:50 Western blot: 1:1,000-1:2,000 *Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Store at 4°C. Do not freeze.		

Application Notes

In Immunofluorescence/immunocytochemistry, cytoplasmic and membrane staining was observed in HeLa cells.

Western Blot:

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 20 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS for 2 hours at room temperature.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-Cripto (human) primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers' instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers' instructions (Pierce's ECL).

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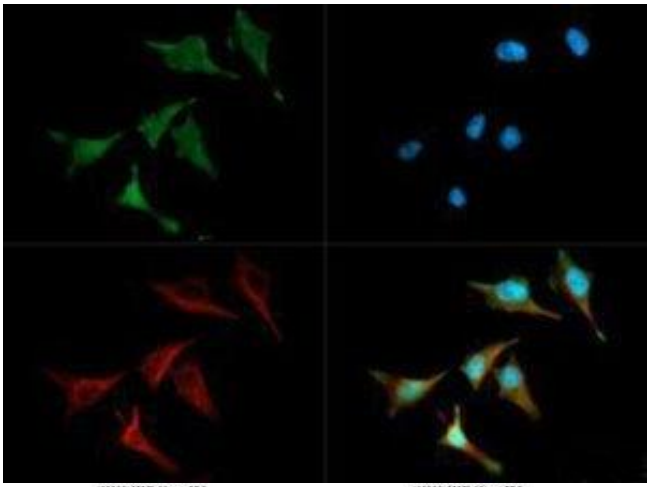
Images:Immunocytochemistry/Immunofluorescence: Cripto1 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei were counterstained with DAPI (blue) and tubulin was stained with alpha tubulin (red).



Images: FACS staining of NTERA-2 cells using Cripto1 antibody at a 1:50 dilution detected using Dylight-488 conjugated goat anti-rabbit IgG secondary

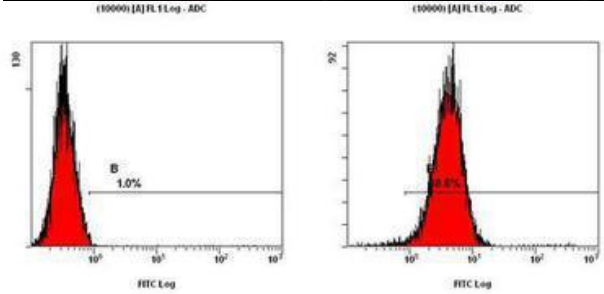
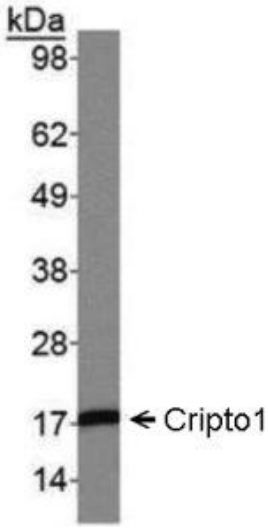


Image: Western blot analysis of Cripto1 in MDA-MB231 lysates.



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