NEUROMICS

c-Myc Data Sheet

Catalog Number:	RA25085	**Host:**	Rabbit
Product Type:	Affinity Purified	**Species Reactivity:**	Human
Immunogen Sequence:	Human c-myc (EQKLISEEDL) conjugated to KLH.	**Format:**	Liquid. Tris-glycine, 150mM NaCl and 0.09% Sodium Azide as a preservative. Concentration: 1.0 mg/ml.

Applications: Immunofluorescence: 1:50-1:200
Western blot: 1:1000

*Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Store frozen. Aliquot as undiluted antisera and immediately place at -20°C. Antisera may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20°C. Repeated freeze/thaw cycles compromise the integrity of the antiserum.

Publications:

1. Tang CJ, Fu RH, Wu KS, et al. CPAP is a cell-cycle regulated protein that controls centriole length. Nat Cell Biol. 2009 Jul;11(7):825-31. [PMID: 19503075]
2. Sun Y, et al. Rab6 regulates both ZW10/RINT-1 and conserved oligomeric Golgi complex-dependent Golgi trafficking and homeostasis. Mol Biol Cell. 2007 Oct;18(10):4129-42. Epub 2007 Aug 15.

Application Notes

Immunofluorescence staining pattern is Cytoplasmic and Nuclear.

Western Blot:

Sample Preparation

1. Remove media and wash cells with sterile PBS.
2. Scrape cells into PBS and pellet in a centrifuge.
3. Resuspend the pellet in 0.3 mL of RIPA buffer.
4. Pass lysate through a 21-guage needle 3-4 times to shear the DNA.
5. Incubate on ice for 30-60 minutes.
6. Spin at 15,000g for 20 minutes at 4 degrees Celcius.
7. Remove and siphon the supernatant (keep).
8. Measure the protein content in the supernatant.
9. Add loading buffer and B-mercaptoethanol or DTT to the supernatant.
10. Boil samples for 3-5 minutes at 95 degrees Celcius (unless noted otherwise).

Western Blotting

1. Block the membrane for 1-2 hours in Block (5% NFDM, 1% BSA in TBS / 0.1% Tween) at RT. After this step you can either proceed to primary or you can rinse with dH20 and dry your membrane.
2. Cut the blot into the appropriate strips or keep whole and incubate with your primary antibody diluted to the appropriate concentration in 5 – 15ml of Block. (Generally, antibodies are tested at 0.5 and 2.0 µg/ml initially.) Incubate overnight at 4°C on a shaker.
3. Rinse the blot once separately with TBST, and then wash vigorously 5 x 5 minutes in TBST in a tray.
4. Dilute the appropriate secondary antibody in 25ml Block and incubate 1 hour at R.T. (Anti-Rabbit HRP 1:25000, anti-Mouse HRP 1:10,000 typically)

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5. Rinse the blot once with TBST; wash vigorously 5x 5 minutes in TBST. Rinse blot with dH2O before adding to ECL.Make up fresh ECL (1:1) or according to the manufacturer's instructions. Incubate blot for 5 minutes.
6. Blot end of each strip on a Kim wipe to wick off excess ECL solution and assemble blot. Be sure to get out the excess liquid when finished and dry off ends. Be careful to never let blot become dry as this increases background greatly.

Image: Detection of c-myc Tagged Plakoglobin by Immunofluorescence. Samples: Human microvascular endothelial cells expressing c-myc tagged plakoglobin following transient transfection.

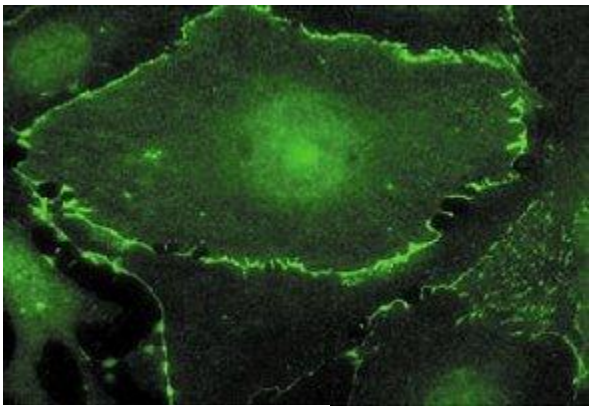
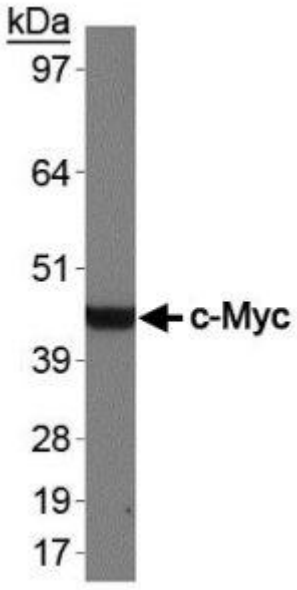


Image: Western blot analysis of c-Myc on Jurkat whole cell extract.



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