NEUROMICS

Ubiquitin +1 Data Sheet

Catalog Number:	RA15043	**Host:**	Rabbit
Product Type:	Affinity purified	**Species Reactivity:**	Human
Immunogen Sequence:	(C)ADLREDPDRQDHHPGSGAQ	**Format:**	Liquid 1mg/ml 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Applications:

Immunohistochemistry: 15 µg/mL

Western Blot: 1 µg/mL
Tested for Western blotting using recombinant human Ubiquitin +1.
DOES NO CROSS REACT WITH UBIQUITIN

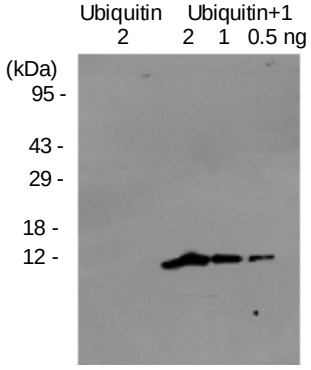
Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. *Avoid repeated freeze-thaw cycles.*

Application Notes

Western blotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4 0.15 M NaCl 0.1% Tween 20	5% nonfat dry milk in Blotting Buffer, Adjust pH to 7.4	5% nonfat dry milk in Blotting Buffer Adjust pH to 7.4



Immunoblots of 2 ng recombinant human Ubiquitin and 2, 1, and 0.5 ng recombinant human Ubiquitin+1. Samples were electrophoresed on 15% gels and immunoblotting was with 1.0 µg/mL anti-Ubiquitin+1. A one minute exposure is shown.

1. Transfer the electrophoresed proteins to Immobilon filters (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 0.1µg/mL rabbit anti-Ubiquitin+1.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a HRP-conjugated anti-rabbit IgG secondary antibody.
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with ECL Reagent (Amersham).

Cell lysates for Western blottings: To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at 2 x 10_6 - 1 x 10_7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

Immunohistochemistry

The antibody will detect Ubiquitin+1 in paraffin-embedded tissue sections. The working dilution after antigen retrieval is 15 µg/mL.

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