NEUROMICS

Ubiquitin+1 Data Sheet

Catalog Number:	RA15043	**Host:**	Rabbit
Product Type:	KLH coupled synthetic peptide CADLREDPDRQDHHPGSGAQ. The sequence corresponds to the carboxyl terminus of Ubiquitin+1 (van Leeuwen, F.W. et al., 1998, Science 279:242). Cysteine was added to the amino-terminus for conjugation to KLH and for coupling to an affinity matrix.	**Species Reactivity:**	Human
Immunogen Sequence:	Hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with rat brain synaptic plasma membranes.	**Format:**	Liquid 1mg/ml Solution in phosphate-buffered saline (PBS) with 5% Trehlose
Applications:	Immunohistochemistry -3-25 µg/mL Immunoprecipitation-See reference Western Blot-See reference		

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

Storage: Antibody can 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

Specificity
The antibody detects human Ubiquitin+1. It does not cross-react with Ubiquitin.

Western blotting

Blotting buffer	*Blocking solution*	*Antibody solution*
25 mM Tris, pH 7.5 0.15 M NaCl 0.05% Tween 20	2% nonfat dry milk in blotting buffer pH to 7.5	1% nonfat dry milk in blotting buffer pH to 7.5

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 in antibody solution containing 1.0 µg/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 for 1 hour at room temperature in antibody solution containing a 1:2,000 dilution of HRP-conjugated Protein A (Amersham).
5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
6. Detection was with ECL Reagent (Amersham).

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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, and bromophenyl blue) at 2 x 106 - 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

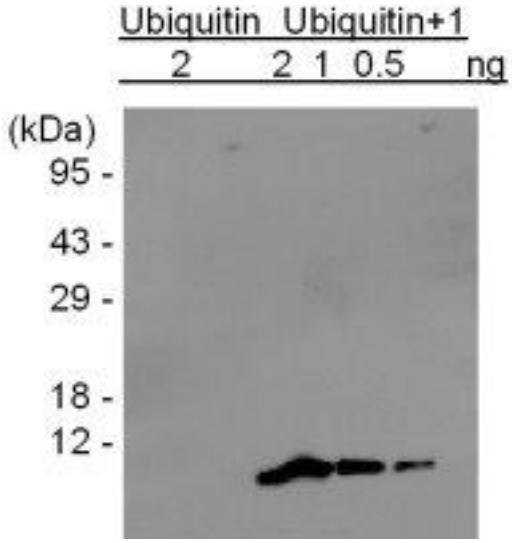

Image: Immunoblots of 2 ng recombinant human Ubiquitin and 2, 1, and 0.5 ng recombinant human Ubiquitin+1. Samples were electrophoresed on 15% gels and immunoblotting was with 1.0 µg/mL anti-Ubiquitin+1. A one minute exposure is shown.

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