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Product datasheet for AM26675PU-N

Ubiquitin (UBB) Mouse Monoclonal Antibody [Clone ID: 2C5]

Product data:

Product Type:	Primary Antibodies
Clone Name:	2C5
Applications:	WB
Recommended Dilution:	Western blot: 5 µg/mL for chemiluminescence detection system. For details see protocol below.
Reactivity:	Bovine, Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Bovine erythrocyte ubiquitin
Specificity:	This antibody reacts with erythrocyte ubiquitin. Clone 2C5 does not react with multi ubiquitin.
Formulation:	PBS (pH 7.2)/ 1% sucrose State: Purified State: Lyophilized Ig fraction
Reconstitution Method:	Prepare a stock solution by dissolving the lyophilized antibody in 100 µl of distilled water. After reconstitution, the IgG concentration will be 1 mg/ml.
Concentration:	lot specific
Purification:	Ammonium sulfate precipitation and protein A agarose
Conjugation:	Unconjugated
Storage:	Prior to reconstitution store at 2-8°C. Following reconstitution store (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	ubiquitin B
Database Link:	Entrez Gene 7314 Human P0CG47

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Background: Ubiquitin is a polypeptide of 76 amino acid residues, and widely distributed protein in eukaryotic cells. This protein is also highly conserved among eukaryotic cells. There are several reports showed that intracellular abnormal and short-lived proteins are degraded through an ubiquitin dependent proteolytic pathway. In the ubiquitin dependent pathway, a target protein is tagged with multi-ubiquitin molecules.

Synonyms: UBB, Polyubiquitin-B

Note: This product was originally produced by MBL International.

Protocol:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4oC with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4oC and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4oC.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.
(Positive controls for Western blotting; Jurkat, HeLa, WR19L, PC12)