

**Catalog No. PEN-MA001****Anti Puromycin [Clone : 3RH11]**

## Background :

Puromycin is an aminonucleoside antibiotic, derived from the *Streptomyces alboniger* bacterium, that causes premature chain termination during translation taking place in the ribosome. Part of the molecule resembles the 3' end of the aminoacylated tRNA, making it useful for protein translation analysis.

Classical pulse-chase or flooding dose methods used to monitor protein synthesis rely on the measurement of radioactive methionine and cysteine labels. Analysis using puromycin immunodetection is an advantageous alternative to radioactive amino acid labeling, and allows for the evaluation/quantification of translation directly using standard immunochemical methods.

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|----------------------|--|
| Applications:        | Western Blotting (1:1,000), ELISA (1:10,000) and Immunofluorescence microscopy |
| Specificity:         | Puromycin  |
| Immunogen:           | Puromycin hydrochloride  |
| Host:                | Mouse  |
| Clonality:           | Monoclonal (clone# 3RH11)  |
| Subclass:            | IgG1 Kappa   |
| Purification method: | Purified from cell culture medium by affinity column (Protein G)               |
| Form:                | Liquid (PBS), no preservatives added   |
| Volume:              | 100 ug   |
| Concentration:       | 1 mg/mL  |
| Storage condition:   | -20°C  |

## References:

1: Kelleher AR, Gordon BS, Kimball SR, Jefferson LS. Changes in REDD1, REDD2, and atrogene mRNA expression are prevented in skeletal muscle fixed in a stretched position during hindlimb immobilization.

Physiol Rep. 2014 Feb 25;2(2):e00246. doi: 10.1002/phy2.246. eCollection 2014 Feb 1.

PubMed PMID: 24744910; PubMed Central PMCID: PMC3966240.

2: Kelleher AR, Kimball SR, Dennis MD, Schilder RJ, Jefferson LS. The mTORC1 signaling repressors REDD1/2 are rapidly induced and activation of p70S6K1 by leucine is defective in skeletal muscle of an immobilized rat hindlimb.

Am J Physiol Endocrinol Metab. 2013 Jan 15;304(2):E229-36. doi: 10.1152/ajpendo.00409.2012. Epub 2012 Nov 27.

PubMed PMID: 23193052; PubMed Central PMCID: PMC3543567.

Example Assay Data:

1. Cell Culture

Human fibroblast (TIG-1 cells) was plated to 12-well plate (Cells were cultured till they became 80% confluent).

Puromycin (final conc. 0 uM and 5 uM) was added to the medium of the cells 80 min before harvest.

Wells were washed with PBS, followed by addition of 1mL buffer (20 mM Tris-HCl(pH8.0)+Protease Inhibitor) to each wells to perform ultrasonic fragmentation.

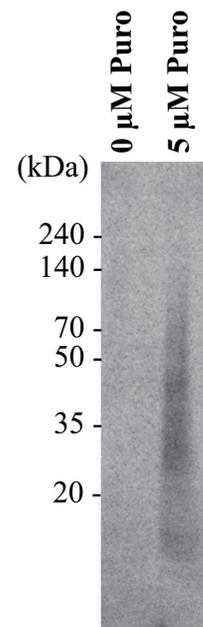
Cells were spun down (4°C, 12,000 g, 20 min), supernatant was collected for downstream assays (Western Blotting, ELISA).

2. Western Blotting

Loaded 15 ug/lane

Primary Antibody (anti puromycin [CAC-PEN-MA001]) 1:1,000 dilution (1 ug/mL)

Secondary Antibody (anti mouse Ig-HRP, DAKO, Cat.No. P0161) 1:2,000 dilution



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