

# Anti-Urm1 (RABBIT) Antibody

**Urm1 Antibody** Catalog # ASR4398

#### **Specification**

## Anti-Urm1 (RABBIT) Antibody - Product Information

Host Rabbit

Conjugate Unconjugated **Target Species** Yeast Reactivity Yeast Clonality **Polyclonal** Application WB, E, I, LCI

**Application Note** This purified polyclonal antibody reacts with yeast Urm1 by western blot and

ELISA. Although not tested, this antibody

is likely functional in

immunohistochemistry and

immunoprecipitation. This antibody using the specified conditions may recognize other prominent intrinsic bands (UBLs or their conjugates). Other intrinsic bands are readily detectable in yeast lysates at

lower antibody dilutions. For immunoblotting a 12 kDa band

corresponding to yeast Urm1 is detected. Most yeast cell lysates can be used as a positive control without induction or

stimulation.

Lvophilized

0.02 M Potassium Phosphate, 0.15 M

Sodium Chloride, pH 7.2

This purified antibody was prepared from **Immunogen** 

rabbit serum after repeated immunizations

with recombinant yeast Urm1 protein.

Reconstitution Volume 100 µL

Reconstitution Buffer Restore with deionized water (or

equivalent)

Preservative 0.01% (w/v) Sodium Azide

# Anti-Urm1 (RABBIT) Antibody - Additional Information

**Gene ID 854809** 

**Other Names** 854809

**Physical State** 

Buffer

This product is an IgG fraction antibody purified from monospecific antiserum by a multi-step process which includes delipidation, salt fractionation and ion exchange chromatography followed by extensive dialysis against the buffer stated above. Assay by immunoelectrophoresis resulted in



a single precipitin arc against anti-Rabbit Serum.

#### **Storage Condition**

Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

#### **Precautions Note**

This product is for research use only and is not intended for therapeutic or diagnostic applications.

#### Anti-Urm1 (RABBIT) Antibody - Protein Information

Name URM1 {ECO:0000255|HAMAP-Rule:MF\_03048}

#### **Function**

Acts as a sulfur carrier required for 2-thiolation of mcm(5)S(2)U at tRNA wobble positions of cytosolic tRNA(Lys), tRNA(Glu) and tRNA(Gln). Serves as sulfur donor in tRNA 2-thiolation reaction by being thiocarboxylated (-COSH) at its C-terminus by the MOCS3 homolog UBA4. The sulfur is then transferred to tRNA to form 2-thiolation of mcm(5)S(2)U. Prior mcm(5) tRNA modification by the elongator complex is required for 2-thiolation (By similarity) (PubMed:<a href="http://www.uniprot.org/citations/18664566" target=" blank">18664566</a>, PubMed:<a href="http://www.uniprot.org/citations/18755837" target="blank">18755837</a>, PubMed:<a href="http://www.uniprot.org/citations/19017811" target=" blank">19017811</a>, PubMed:<a href="http://www.uniprot.org/citations/19145231" target="\_blank">19145231</a>, PubMed:<a href="http://www.uniprot.org/citations/19151091" target="\_blank">19151091</a>). Also acts as a ubiquitin-like protein (UBL) that is covalently conjugated via an isopeptide bond to lysine residues of target proteins such as AHP1 (PubMed:<a href="http://www.uniprot.org/citations/10713047" target=" blank">10713047</a>, PubMed:<a href="http://www.uniprot.org/citations/14551258" target="blank">14551258</a>, PubMed:<a href="http://www.uniprot.org/citations/14555475" target="blank">14555475</a>, PubMed:<a href="http://www.uniprot.org/citations/21209336" target="blank">21209336</a>, PubMed:<a href="http://www.uniprot.org/citations/32004955" target="blank">32004955</a>). Conjugation does not depend on the canonical cascade of E2 ubiquitin-conjugating enzymes and/or E3 ligases. The conjugation reaction requires a thiocarboxylated C- terminus of URM1 and a peroxidatic cysteine in the target protein, as the sulfur atom of the URM1 thiocarboxyl group is transferred to redox- active cysteine residues in the target protein (PubMed: <a href="http://www.uniprot.org/citations/36102610" target=" blank">36102610</a>). Oxidative stress specifically induces the formation of UBL-protein conjugates (PubMed: <a href="http://www.uniprot.org/citations/21209336" target=" blank">21209336</a>). Covalent modification with URM1 promotes the phase separation of a wide range of proteins into condensates like stress granules (PubMed: <a href="http://www.uniprot.org/citations/38942013" target=" blank">38942013</a>). Indirectly involved in regulation of budding and haploid invasive growth (PubMed:<a href="http://www.uniprot.org/citations/14551258" target=" blank">14551258</a>).

#### **Cellular Location**

Cytoplasm. Nucleus.

## Anti-Urm1 (RABBIT) Antibody - Protocols

Provided below are standard protocols that you may find useful for product applications.



- Western Blot
- Blocking Peptides
- Dot Blot
- <u>Immunohistochemistry</u>
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

## Anti-Urm1 (RABBIT) Antibody - Images



Western blot of Urm1 fusion protein. Anti- Urm1 antibody generated by immunization with recombinant yeast Urm1 was tested by western blot against yeast lysates expressing the Urm1-GFP fusion protein and other UBL fusion proteins. All UBLs possess limited homology to Ubiquitin and to each other, therefore it is important to know the degree of reactivity of each antibody against each UBL. Panel A shows total protein staining using ponceau. Panel B shows positions of free GFP or GFP containing recombinant proteins present in each lysate preparation after reaction with a 1:1,000 dilution of Rockland's anti-GFP (code # 600-101-215) followed by reaction with a 1:15,000 dilution of HRP Donkey-a-Goat IgG MX (code # 605-703-125). Panel C shows specific reaction with Urm1 using a 1:1,000 dilution of Rockland's IgG fraction of Rabbit-anti- Urm1 (Yeast) followed by reaction with a 1:15,000 dilution of HRP Goat-a-Rabbit IgG MX (code # 611-103-122). All primary antibodies were diluted in TTBS buffer supplemented with 5% non-fat milk and incubated with the membranes overnight at 4° C. Yeast lysate proteins were separated by SDS-PAGE using a 15% gel. This data indicates that anti-Urm1 is highly specific and does not cross react with other UBLs. Bands present in Panel C indicate that Urm1 and conjugated Urm1 is present in most yeast cell lysates albeit at significantly reduced levels relative to the Urm1-GFP transfected lysate. A chemiluminescence system was used for signal detection (Roche). Other detection systems will yield similar results. Data contributed by M. Malakhov, www.lifesensors.com, personal communication.

#### Anti-Urm1 (RABBIT) Antibody - Background

Ubiquitin-like proteins fall into two classes: the first class, ubiquitin-like modifiers (UBLs) function as modifiers in a manner analogous to that of ubiquitin. Examples of UBLs are SUMO, Rub1 (also called Nedd8), Apg8 and Apg12. Proteins of the second class include parkin, RAD23 and DSK2, are designated ubiquitin-domain proteins (UDPs). These proteins contain domains that are related to ubiquitin but are otherwise unrelated to each other. In contrast to UBLs, UDPs are not conjugated to other proteins. Urm1 is a newly identified ubiquitin related modifier. Urm 1 is a 99-amino acid protein terminated with glycine-glycine. Target proteins are conjugated to Urm1 via its C-terminal glycine. Initially Urm1 forms a thioester with a novel E1-like protein, Uba4.