

# **Anti-UBIQUITIN (RABBIT) Antibody**

Ubiquitin Antibody Catalog # ASR4391

### **Specification**

# **Anti-UBIQUITIN (RABBIT) Antibody - Product Information**

Host Rabbit

Conjugate Unconjugated Target Species Rabbit Clonality Polyclonal

Application WB, IHC, E, I, LCI

Application Note This purified polyclonal antibody reacts

with ubiquitin by ELISA and western blot. Although not tested, this antibody is likely functional in immunohistochemistry and immunoprecipitation. For detection of free Ub by western blotting use Tris-Tricine SDS-PAGE and autoclaved nitrocellulose filters after the transfer and before blocking and addition of anti-Ub

antibodies. Details on western blotting procedures are found in Mimnaugh et al.,

(1999 and 2002).

Physical State Lyophilized

Buffer 0.02 M Potassium Phosphate, 0.15 M

Sodium Chloride, pH 7.2

Immunogen This purified antibody was prepared from

rabbit serum after repeated immunizations with ubiquitin coupled to rabbit IgG using

glutaraldehyde.

Reconstitution Volume 100 μL

Reconstitution Buffer Restore with deionized water (or

equivalent)

Preservative 0.01% (w/v) Sodium Azide

## Anti-UBIQUITIN (RABBIT) Antibody - Additional Information

**Gene ID 7314** 

**Other Names** 

7314

#### Purity

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-UBIQUITIN (RABBIT) Antibody - Protein Information

#### Name UBB

### **Function**

[Ubiquitin]: Exists either covalently attached to another protein, or free (unanchored). When covalently bound, it is conjugated to target proteins via an isopeptide bond either as a monomer (monoubiquitin), a polymer linked via different Lys residues of the ubiquitin (polyubiquitin chains) or a linear polymer linked via the initiator Met of the ubiquitin (linear polyubiquitin chains). Polyubiquitin chains, when attached to a target protein, have different functions depending on the Lys residue of the ubiquitin that is linked: Lys-6-linked may be involved in DNA repair; Lys-11-linked is involved in ERAD (endoplasmic reticulum-associated degradation) and in cell-cycle regulation; Lys-29-linked is involved in proteotoxic stress response and cell cycle; Lys-33-linked is involved in kinase modification; Lys-48-linked is involved in protein degradation via the proteasome; Lys-63-linked is involved in endocytosis, DNA-damage responses as well as in signaling processes leading to activation of the transcription factor NF-kappa-B. Linear polymer chains formed via attachment by the initiator Met lead to cell signaling. Ubiquitin is usually conjugated to Lys residues of target proteins, however, in rare cases, conjugation to Cys or Ser residues has been observed. When polyubiquitin is free (unanchored-polyubiquitin), it also has distinct roles, such as in activation of protein kinases, and in signaling.

### **Cellular Location**

[Ubiquitin]: Cytoplasm. Nucleus. Mitochondrion outer membrane; Peripheral membrane protein

#### **Anti-UBIQUITIN (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
- <u>Immunoprecipitation</u>
- Flow Cytomety
- Cell Culture

# Anti-UBIQUITIN (RABBIT) Antibody - Images





Anti-Ubiquitin antibody, generated by immunization with Ubiquitin coupled to Rabbit IgG, was tested by western blot against total cell extract from yeast. Dilution of the antibody between 1:200 and 1:1,000 showed strong reactivity with Ubiquitinated proteins. In this blot the antibody was used at a 1:500 dilution incubated overnight at 4° C in 5% non-fat dry milk in TTBS. Detection occurred using a 1:2000 dilution of HRP-labeled Donkey anti-Rabbit IgG (code # 611-703-127) for 1 hour at room temperature. A chemi-luminescence system was used for signal detection (Roche). Other detection systems will yield similar results.

# Anti-UBIQUITIN (RABBIT) Antibody - Background

Ubiquitin (Ub) is a small, 76-residue, protein (8.5 kDa) found both as free monomer and covalently attached to itself and other proteins in eukaryotic cells. Free Ub is a very compact and stable molecule that is easily refolded after being denatured. It is therefore recommended that for detection of free Ub on Westerns, the Tris-Tricine SDS-PAGE is used and nitrocellulose filters are autoclaved after the transfer and before blocking and addition of anti-Ub antibodies. The C-terminus of ubiquitin forms an isopeptide bond with the e-amino group of a lysine side chain in a target protein. In this way proteins can be covalently modified by the addition of ubiquitin which may alter the target protein's function. Monoubiquitination generally targets proteins for internalization, endocytosis and lysosomal degradation, or modifies the surface charge of histones and affects chromatin compaction. Conjugation of ubiquitin (Ub) involves a three-step mechanism whereby specific enzymes (or enzyme complexes) activate and covalently link Ub to their substrates.