Product Specification Sheet

Product: IgG fraction of Anti-UBIQUITIN (Rabbit)

Code: 200-401-431

Lot #: 12731

Size: 500 g

Antibody Concentration: 5.0 mg/ml (by UV absorbance at 280 nm)

Stabilizer: None

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Preservative: 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at 4° C prior to restoration. Restore with 0.1 ml of deionized water (or equivalent). 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. Expiration date is one (1) year from date of restoration.

Background Information: 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 -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. Multi-ubiquitin chains can be built up on a single lysine of the target protein, by isopeptide bond formation usually between the carboxyl group of Gly76 of one Ub with the amino group of the side chain of Lys of the preceding ubiquitin. Although Lys48 of Ub is most frequently used for multi-ubiquitin chain formation, other lysines have also been shown to form bonds with Gly76. If a chain of multiple copies of ubiquitin is attached to a protein, this appears to target the protein for degradation by the large intacellular protease known as the 26S proteasome. The process of intracellular proteasomal proteolysis is very rapid and efficient. This makes detection of certain ubiquitinated proteins extremely difficult. Several specific inhibitors of proteasomes are commercially available and have been proven to be very useful in research applications. The detection of ubiquitinated species is further complicated by the fact that the process of ubiquitination is reversible and is accomplished by highly specific proteases present in all eukaryotic cells. It is therefore recommended that cell lysates are kept on ice and the lysis buffers contain one or more of cysteine protease inhibitors (n-ethylmaleimide, E64, etc.).

Application Note(s): This purified polyclonal antibody reacts with Ubiquitin 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 conjugates). Other intrinsic bands are readily detectable at lower dilutions. Details on western blotting procedures are found in Mimnaugh et al., (1999 and 2002).

Purity and Specificity: 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.

Immunogen: This purified antibody was prepared from rabbit serum after repeated immunizations with ubiquitin coupled to rabbit IgG.

Reference(s):
Mimnaugh, E.G., Bovini, P. and Neckers, L. (1999) The measurement of ubiquitin and ubiquinated proteins. *Electrophoresis* **2**0:418-428.
Wilkinson, K.D. (2000) Ubiquination and deubiquination: targeting of proteins for degradation by the proteasome. *Cell Dev. Bio*.**1**1:141-148.
Wilkinson, K. D., and Hochstrasser, M. (1998) in *Ubiquitin and theBiology of the Cell* (Peters, J. M. , Harris, J. R. , and Finley, D., eds) , pp. 99-125, Plenum Press, New York.
Mimnaugh, E.G., Neckers, L.M. (2002) Immunoblotting methods for the study of protein ubiquitination. *Methods Mol Biol.* **19**4:179-203.
Liakopoulos D et al. (1998). A novel protein modification pathway related to the ubiquitin syste*m. EMBO J.* 15;**17**(8):2208-14.
Jentsch S, Pyrowolakis G. (2000) Ubiquitin and its kin: how close are the family ties? *Trends Cell Bio*l. **1**0(8):335-42.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.