

#### Ubiquitin Antibody

Ubiquitin Antibody, Clone 5B9-B3 Catalog # ASM10087

#### Specification

### **Ubiquitin Antibody - Product Information**

WB Application **Primary Accession** P0CG53 NP 776558.1 Other Accession Host Mouse Isotype IgG2a Kappa Human, Mouse, Rat, Bovine Reactivity **Monoclonal** Clonality Description Mouse Anti-Bovine Ubiguitin Monoclonal IgG2a Kappa

**Target/Specificity** Detects ~10kDa.

**Other Names** Polyubiquitin B Antibody, RPS27A Antibody, UBA52 Antibody, UBB Antibody, UBC Antibody, ubiquitin B Antibody

Immunogen Native bovine ubiquitin, conjugated to KLH

**Purification** Protein G Purified

Storage Storage Buffer PBS pH7.4, 50% glycerol, 0.09% sodium azide -20ºC

Shipping TemperatureBlue Ice or 4°CCertificate of Analysis1 μg/ml of SMC-160 was sufficient for detection of ubiquitin in 10 μg of Heal Lysate by colorimetricimmunoblot analysis using Goat anti-mouse IgG:HRP as the secondary antibody.

Cellular Localization Cytoplasm | Nucleus

# **Ubiquitin Antibody - Protocols**

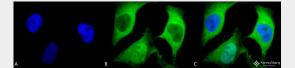
Provided below are standard protocols that you may find useful for product applications.

- <u>Western Blot</u>
- Blocking Peptides
- <u>Dot Blot</u>
- Immunohistochemistry

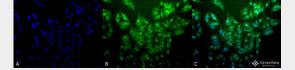


- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- <u>Cell Culture</u>

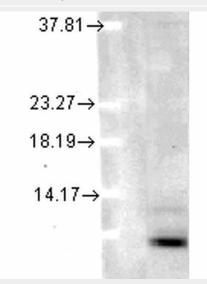
# **Ubiquitin Antibody - Images**



Immunocytochemistry/Immunofluorescence analysis using Mouse Anti-Ubiquitin Monoclonal Antibody, Clone 5B9-B3 (ASM10087). Tissue: HeLa Cells. Species: Human. Fixation: 2% Formaldehyde for 20 min at RT. Primary Antibody: Mouse Anti-Ubiquitin Monoclonal Antibody (ASM10087) at 1:100 for 12 hours at 4°C. Secondary Antibody: FITC Goat Anti-Mouse (green) at 1:200 for 2 hours at RT. Counterstain: DAPI (blue) nuclear stain at 1:40000 for 2 hours at RT. Localization: Diffuse nuclear and cytoplasmic staining. Magnification: 100x. (A) DAPI (blue) nuclear stain. (B) Anti-Ubiquitin Antibody. (C) Composite.



Immunocytochemistry/Immunofluorescence analysis using Mouse Anti-Ubiquitin Monoclonal Antibody, Clone 5B9-B3 (ASM10087). Tissue: HeLa Cells. Species: Human. Fixation: 2% Formaldehyde for 20 min at RT. Primary Antibody: Mouse Anti-Ubiquitin Monoclonal Antibody (ASM10087) at 1:100 for 12 hours at 4°C. Secondary Antibody: FITC Goat Anti-Mouse (green) at 1:200 for 2 hours at RT. Counterstain: DAPI (blue) nuclear stain at 1:40000 for 2 hours at RT. Localization: Diffuse nuclear and cytoplasmic staining. Magnification: 20x. (A) DAPI (blue) nuclear stain. (B) Anti-Ubiquitin Antibody. (C) Composite.



Western Blot analysis of Human cell lysates showing detection of Ubiquitin protein using Mouse Anti-Ubiquitin Monoclonal Antibody, Clone 5B9-B3 (ASM10087). Load: 15  $\mu$ g. Block: 1.5% BSA for 30 minutes at RT. Primary Antibody: Mouse Anti-Ubiquitin Monoclonal Antibody (ASM10087) at 1:1000 for 2 hours at RT. Secondary Antibody: Sheep Anti-Mouse IgG: HRP for 1 hour at RT.

#### Ubiquitin Antibody - Background



Ubiguitin is a small protein that occurs in all eukaryotic cells. The ubiguitin protein itself consists of 76 amino acids and has a molecular mass of about 8.5kDa. Key features include its C-terminal tail and the 7 Lys residues. It is highly conserved among eukaryotic species: Human and yeast ubiquitin share 96% sequence identity (1). The main function of Ubiguitin is to clear abnormal, foreign and improperly folded proteins by targeting them for degradation by the 26S proteosome (2). Ubiquitination represents an essential cellular process affected by a multi-enzyme cascade involving classes of enzymes known as ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s or Ubcs) and ubiguitin-protein ligases (E3s). Ubiguitin is activated in a two-step reaction by an E1 ubiguitin-activating enzyme in a process requiring ATP as an energy source. The initial step involves production of an ubiquitin-adenylate intermediate. The second step transfers ubiquitin to the E1 active site cysteine residue, with release of AMP. This step results in a thioester linkage between the C-terminal carboxyl group of ubiquitin and the E1 cysteine sulfhydryl group. The third step is a transfer of ubiguitin from E1 to the active site cysteine of a ubiguitin-conjugating enzyme E2 via a trans(thio)esterification reaction. And the final step of the ubiquitylation cascade creates an isopeptide bond between a lysine of the target protein and the C-terminal glycine of ubiquitin. In general, this step requires the activity of one of the hundreds of E3 ubiquitin-protein ligases (often termed simply ubiguitin ligase). E3 enzymes function as the substrate recognition modules of the system and are capable of interaction with both E2 and substrate(2, 3). Ubiguitination also participates in the internalization and degradation of plasma membrane proteins such as some of the TCR subunits while still ER-membrane associated (4). Ubiguitin also plays a role in regulating signal transduction cascades through the elimination inhibitory proteins, such as IkBa and p27 (5).

# **Ubiquitin Antibody - References**

- 1. Wilkinson K.D. (1995) Annu. Rev. Nutr. 15:161-189.
- 2. Bonifacino J.S., et al. (1998) Annu Rev Cell Dev Biol. 14: 19-57.
- 3. Boston Biochem: "Ubiquitin Proteasome Pathway Overview"
- http://www.bostonbiochem.com/upp.php
- 4. Yang M., et al. (1998) J Exp Med. 187: 1835-1846.
- 5. Chen Z.J., et al. (1996) Cell 84: 853-862.