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## MATERIAL DATA SHEET

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### Recombinant Human GST Ubiquitin

#### Cat. # U-540

Ubiquitin is a 76 amino acid (aa) protein that is ubiquitously expressed in all eukaryotic organisms. Ubiquitin is highly conserved with 96% aa sequence identity shared between human and yeast Ubiquitin, and 100% aa sequence identity shared between human and mouse Ubiquitin (1). In mammals, four Ubiquitin genes encode for two Ubiquitin-ribosomal fusion proteins and two poly-Ubiquitin proteins. Cleavage of the Ubiquitin precursors by deubiquitinating enzymes gives rise to identical Ubiquitin monomers each with a predicted molecular weight of 8.6 kDa. Conjugation of Ubiquitin to target proteins involves the formation of an isopeptide bond between the C-terminal glycine residue of Ubiquitin and a lysine residue in the target protein. This process of conjugation, referred to as ubiquitination or ubiquitylation, is a multi-step process that requires three enzymes: a Ubiquitin-activating (E1) enzyme, a Ubiquitin-conjugating (E2) enzyme, and a Ubiquitin ligase (E3). Ubiquitination is classically recognized as a mechanism to target proteins for degradation and as a result, Ubiquitin was originally named ATP-dependent Proteolysis Factor 1 (APF-1) (2,3). In addition to protein degradation, ubiquitination has been shown to mediate a variety of biological processes such as signal transduction, endocytosis, and post-endocytic sorting (4-7).

Fully functional Ubiquitin with an N-terminal GST-tag (Glutathione S-transferase) allows for Glutathione affinity purification of ubiquitinated molecules and allows for immunodetection of conjugates using GST antibodies. GST-Ub facilitates the visualization of poly-Ubiquitin chains due to larger ladder intervals.

Product Information	
<b>Quantity:</b>	1 mg
<b>MW:</b>	35 kDa
<b>Source:</b>	<i>E. coli</i> -derived Contains an N-terminal GST (glutathione S-transferase) tag Accession # P0CG47
<b>Stock:</b>	Supplied as a solution in HEPES and NaCl.
<b>Purity:</b>	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain.

## Use & Storage

**Use:** Recombinant Human GST- Ubiquitin can be conjugated to substrate proteins via the subsequent actions of a Ubiquitin-activating (E1) enzyme, a Ubiquitin-conjugating (E2) enzyme, and a Ubiquitin ligase (E3). Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human GST- Ubiquitin concentration of 20-100  $\mu$ M.

**Storage:** Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

## Literature

### References:

1. Sharp, P.M. & W.-H. Li. (1987) Trends Ecol. Evol. **2**:328.
2. Ciechanover, A. *et al.* (1980 ) Proc. Natl. Acad. Sci. USA **77**:1365.
3. Hershko, A. *et al.* (1980) Proc. Natl. Acad. Sci. USA **77**:1783.
4. Greene, W. *et al.* (2012) PLoS Pathog. **8**:e1002703.
5. Tong, X. *et al.* (2012) J. Biol. Chem. **287**:25280.
6. Wei, W. *et al.* (2004) Nature **428**:194.
7. Wertz, I.E. *et al.* (2004) Nature **430**:694.

***For research use only. Not for use in humans.***