### Scientific Background
Ubiquitin (Ub) is a highly conserved protein of 8 kDa that covalently attaches to lysine residues of target proteins. This occurs through a three-step process involving Ub-activating (E1), Ub-conjugating (E2) and Ub-ligating (E3) enzymes. Ubiquitin-dependent processes are commonly modulated via formation of polyubiquitin chains, whereby the C terminus of a chain extending ubiquitin becomes linked to the N terminus or one of seven lysine residues (Lys-6, Lys-11, Lys-27, Lys-29, Lys-33, Lys-48, and Lys-63) within a substrate-bound ubiquitin molecule. The modification of lysine ubiquitin (ubiquitination) plays a central role in the regulation of multiple cellular processes. In addition to targeting proteins for proteasomal degradation, ubiquitination modulates membrane protein trafficking, alters protein-protein interactions, and controls the activity of many signal transduction pathways. A large family of enzymes involved in ubiquitin conjugation controls these diverse functions.

### Product Description
The rabbit-derived polyclonal anti-diglycine lysine antibodies (anti-K-ε-G-G antibody) are crosslinked to agarose with stable amide linkages. In the global proteomic screening of lysine ubiquitination, the product was well utilized as affinity matrix to specifically capture the peptides bearing lysine-ε-G-G residues in all species.

### Specificity
With the immobilization of highly specific anti-K-ε-G-G antibody, the diglycine lysine antibody beaded agarose selectively captures peptides/proteins bearing K-ε-G-G residues, but does not cross-react with the peptides bearing other structurally similar modified residues.

### Usage Recommendation
20 μl settled beads per 2 mg peptides, totally 12 preps.

### Related Products:
- Anti-diglycyllysine rabbit pAb
- Anti-ubiquitin rabbit pAb
- Anti-ubiquitin mouse mAb
- Anti-sumo1 2 3 mouse mAb (NT)
- Anti-sumo1 mouse mAb (CT)

### Performance of anti-diglycine lysine antibody conjugated beads

<table>
<thead>
<tr>
<th>Samples</th>
<th>Proteomic summary</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human macrophages (COH-s)</td>
<td>7291 ubiquitinated sites in 2350 proteins</td>
<td>Xiao et al., 2010</td>
</tr>
<tr>
<td>Human fibroblasts (MCF-7)</td>
<td>4944 ubiquitinated sites in 1980 proteins</td>
<td>Xiao et al., 2010</td>
</tr>
<tr>
<td>Plants</td>
<td>1263 ubiquitinated sites in 1011 proteins</td>
<td>Jin et al., 2010</td>
</tr>
</tbody>
</table>

**For research use only, not for therapeutic or diagnostic purposes in humans or animals**
Peptide immunoaffinity enrichment with Antibody Beaded Agarose

1. Mix bead suspension and aliquot 40 µl 50% bead slurry to 0.6 ml tube.

2. Wash beads with 0.5 ml pre-chilled PBS. Spin down beads at 1000 x g for 1 min at 4°C, and remove the supernatants. Repeat twice.

3. Dissolve 2 mg peptides in NETN buffer (100 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, 0.5% Nonidet P-40, pH 8.0).

4. Remove any possible precipitates in peptide solution by centrifuging at 12,000 x g for 10 min at 4°C;

5. Mix peptide solution with pre-washed antibody conjugated beads. Incubate at 4°C for 4h with gentle shaking.

6. Harvest beads by centrifuging at 1000 x g for 1 min at 4°C.

7. Wash the beads four times with 1 ml of NETN buffer and twice with deionized water.

8. Elute bound peptides with 1% trifluoroacetic acid. Repeat twice and combine all three elutes.

(Chen, Y., 2012, Molecular & Cellular Proteomics)

NOTE:
Optimal results could be obtained by using PTM-1104K K-ε-gg peptide enrichment kit (PTM Bio, Inc.).

Outline of peptide immunoaffinity enrichment

1. Extract proteins from samples.

2. Digest proteins to peptide.

3. Dissolve the peptide with NETN buffer, add proper amount of settled antibody beads, incubate at 4°C.

4. Pellet the beads. Wash with NETN Buffer and deionized water step by step.

5. Elute the bound peptides with 1% trifluoroacetic acid.

6. Identify & Quantify peptide by LC-MS/MS

Reference:


