

# Diglycyl-lysine Antibody Clone GX41 Cat. no. 30-2000



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## Product

Anti-diglycyl-lysine antibody (clone GX41) recognizes lysine residues modified by diglycine, an adduct left at sites of ubiquitination after trypsin digestion. Diglycyl-lysine antibody is produced by immunizing animals with a synthetic diglycine-modified histone peptide and established in hybridoma line GX41. Monoclonal antibody is purified by protein A affinity chromatography.

## Applications

Diglycyl-lysine antibody is intended for the detection of proteins containing diglycyl-modified lysines of human, rat, mouse, chicken, cow, horse, and yeast origins by Western blotting and immunoprecipitation.

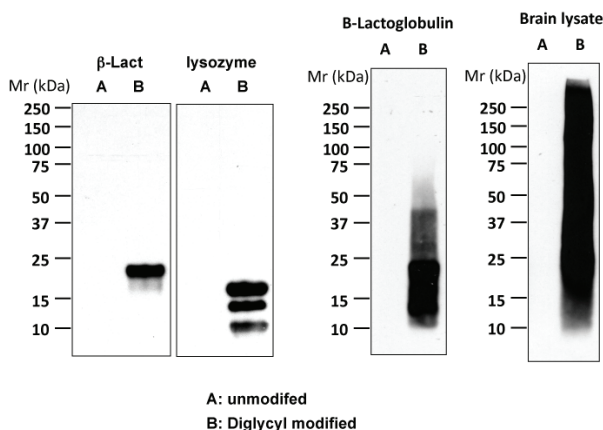
## Presentation

Each vial contains 10 mg lyophilized IgG. Resuspension in 0.2 M HEPES [8.5] at > 1.0 mg/ml concentration is recommended.

## Storage

Store at -20 °C. Stable for 2 years at -20 °C from the date of shipment. Non-hazardous. No MSDS needed.

## Data



Western blot analysis of beta-lactoglobulin, lysozyme, or rat brain lysate, in which the lysines were either unmodified (A) or modified with Gly-Gly (B).

## Protocols (optional)

### Conjugation of GX41 antibody to resin:

Reagents needed:

- \* Affi-gel 10 resin (Bio-Rad, cat. no. 153-6046)
- \* Hydrochloric acid (Fisher, cat. no. A144-500)
- \* Phosphate buffered saline (Fisher, cat. no. BP399-1)
- \* Sodium azide (ICN Biomedicals, cat. no. 102891)
- \* Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl, Invitrogen, cat. no. 15504-20)
- \* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Invitrogen, cat. no. 11344-041)

Procedure:

1. Transfer 50  $\mu$ L of 50 % Affi-gel 10 resin to a 1.6 mL Eppendorf tube.
2. Wash Affi-gel 10 resin twice with 1 mL of ice-cold 1 mM HCl.
3. Add antibody solution to Affi-gel 10 resin and incubate at 25 °C for 4 h or at 4 °C overnight. [Note: Optimal pH is 8.5]
4. Wash beads with 500  $\mu$ L of 1 M Tris-HCl (pH 8.0) and incubate with 500  $\mu$ L of 1 M Tris-HCl solution on an end-over-end rotator at 25 °C for 2 h or at 4 °C overnight.
5. Briefly wash beads twice with 500  $\mu$ L of 0.1 M glycine (pH 2.7) and twice with 0.1 M Tris-HCl (pH 8.0) to neutralize pH.
6. Resuspend Affi-gel 10 resin with PBS containing 0.02 % sodium azide and store at 4 °C.

Notes:

1. Larger batches can be obtained by increasing the quantity of all components proportionally.
2. Alternatively, protocols of coupling antibody to protein G beads can be used here.
3. The immobilized antibody can be kept at 4 °C for more than four weeks.

## Reference

Xu G, Paige JS, Jaffrey SR. 2010. Global analysis of lysine ubiquitination by ubiquitin remnant immunoprecipitation. *Nature Biotechnology* 28, 868-873.