For research use only. Not for use in diagnostic procedures.

POLYCLONAL ANTIBODY

Anti-Glu-Glu-tag pAb

Code No.	Quantity	Form
BRP0001	100 μL	Affinity Purified

BACKGROUND

Epitope tags are short peptide sequences for labeling and detection of targeted proteins. Because of their small size, epitope tags are unlikely to affect the properties of targeted proteins.
Glu-Glu-tag is a commonly used epitope tag. After engineered onto a protein and expression as a fusion protein, the Glu-Glu-tag can be detected using western blot and immunoprecipitation techniques.

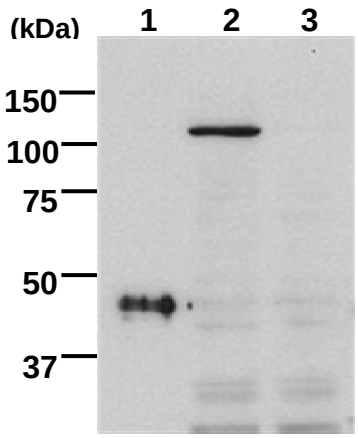
DESCRIPTION

Target	Glu-Glu-tag
Host Species	Rabbit
Clonality	Polyclonal
Immunogen	KLH conjugated Glu-Glu (EEEEYMPME) peptide
Purification	Purified from rabbit serum
Quantity	100 μL
Storage	-20°C
Storage Buffer	PBS containing 50% Glycerol (pH 7.2). No preservatives is contained.

APPLICATIONS

Western blot	1:1000
Immunoprecipitation	5 μL/sample
Immunocytochemistry	Not recommended
Flow cytometry	Not tested

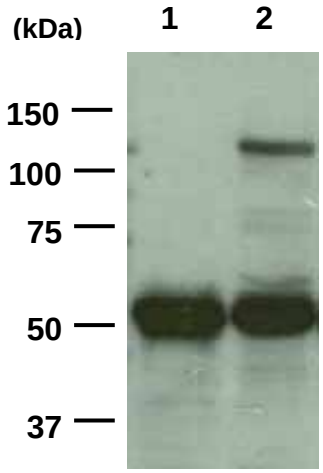
DATA



Western blot analysis of Glu-Glu-tagged protein
Lane 1: Glu-Glu-tagged protein A
Lane 2: Glu-Glu-tagged protein B/293T
Lane 3: 293T

Beijing B&M Biotech Co., Ltd.
Tel: +86-10-8070-7048 **Fax:** +86-10-8289-0976
URL: http://www.bio-med.com.cn/ **E-mail:** info@bio-med.com.cn

For research use only. Not for use in diagnostic procedures.



Immunoprecipitation of Glu-Glu-tagged protein
Lane 1: IP with Normal Rabbit IgG
Lane 2: IP with BRP0001

PROTOCOLS

Western blot

1. For transfectant samples: Harvest 1 x 10^7 cells, wash 3 times with PBS and suspends them in 1 mL of extraction buffer (50 mM Tris-HCl (pH7.5), 150 mM NaCl, 0.05% NP-40), then sonicate briefly (up to 15 seconds). After centrifuged, mix the supernatant with equal volume of Laemmli's sample buffer. For recombinant protein samples: Mix the samples with equal volume of Laemmli's sample buffer.
2. Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane and carry out electrophoresis.
3. Blot the protein to a PVDF membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH).
4. Incubate the membrane in 10% skimmed milk (in PBS, pH 7.2) for at least 1 hour at room temperature or overnight at 4°C.
5. Wash the membrane with PBS-T (0.05% Tween-20 in PBS) for 3 times at least 5 minutes each time on the shaker.
6. Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature.
7. Wash the membrane with PBS-T for 3 times at least 10 minutes each time.
8. Incubate the membrane with the HRP-conjugated anti-Rabbit IgG antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9. Wash the membrane with PBS-T for 3 times at least 10 minutes each time.
10. Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane and seal it in plastic wrap.
11. Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

Beijing B&M Biotech Co., Ltd.
Tel: +86-10-8070-7048 **Fax:** +86-10-8289-0976
URL: http://www.bio-med.com.cn/ **E-mail:** info@bio-med.com.cn

Immunoprecipitation

1. Mix 20 µL of 50% protein A agarose beads slurry resuspended in 200 µL of IP buffer (10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gently agitation for 1 hour at 4°C.
2. Wash the beads 2 times with 1 mL of IP buffer.
3. Add 200 µL of cell lysate of Lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40), then incubate with gentle agitation for 1 hour at 4°C.
4. Wash the beads 6 times with 1 mL of Lysis buffer.
5. Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 2 minutes and centrifuge. Load 20 µL of the sample per lane for electrophoresis.

(See **Western blot**)