

Anti-GRP94 Polyclonal Antibody

Product Details

Ig Type:	IgG
Reactivity:	Human, Mouse, Rat (predicted: Chicken, Dog, Pig, Cow, Horse, Rabbit)
Molecular Weight:	Theoretical: 86 kDa. Actual: 100 kDa.
Purification:	Protein A purified

Applications

1. Paraformaldehyde-fixed, paraffin embedded (rat brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (GRP94) Polyclonal Antibody, Unconjugated (TMAB-06802) at 1: 200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
2. Paraformaldehyde-fixed, paraffin embedded (rat brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (GRP94) Polyclonal Antibody, Unconjugated (TMAB-06802) at 1: 200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
3. Paraformaldehyde-fixed, paraffin embedded (mouse brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (GRP94) Polyclonal Antibody, Unconjugated (TMAB-06802) at 1: 200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
4. Paraformaldehyde-fixed, paraffin embedded (rat brain tissue); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (GRP94) Polyclonal Antibody, Unconjugated (TMAB-06802) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
5. Tissue/cell: human lung carcinoma; 4% Paraformaldehyde-fixed and paraffin-embedded; Antigen retrieval: citrate buffer (0.01 M, pH 6.0), Boiling bathing for 15 min; Block endogenous peroxidase by 3% Hydrogen peroxide for 30 min; Blocking buffer (normal goat serum) at 37°C for 20 min;
Incubation: Anti-GRP94/HSP gp96 Polyclonal Antibody, Unconjugated (TMAB-06802) 1: 200, overnight at 4°C, followed by conjugation to the secondary antibody and DAB staining
6. Sample: Placenta (Mouse) Lysate at 30 µg
Primary: Anti-GRP94 (TMAB-06802) at 1/300 dilution
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/10000 dilution
Predicted band size: 78 kD
Observed band size: 100 kD
7. Blank control: Hela.
Primary Antibody (green line): Rabbit Anti-GRP94 antibody (TMAB-06802)
Dilution: 1 µg/Test;
Secondary Antibody: Goat anti-rabbit IgG-FITC
Dilution: 0.5 µg/Test.
Protocol
The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with

Verified Activity:

90% ice-cold methanol for 20 min at -20°C. The cells were then incubated in 5% BSA to block non-specific protein-protein interactions for 30 min at room temperature. Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.

8. Sample:

Lane 1: Mouse Spleen Lysates

Lane 2: Mouse NIH/3T3 cell Lysates

Primary: Anti-GRP94 (TMAB-06802) at 1/1000 dilution

Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution

Predicted band size: 86 kDa

Observed band size: 100 kDa

9. Paraformaldehyde-fixed, paraffin embedded (human brain glioma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (GRP94) Polyclonal Antibody, Unconjugated (TMAB-06802) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

10. Paraformaldehyde-fixed, paraffin embedded (mouse brain tissue); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (GRP94) Polyclonal Antibody, Unconjugated (TMAB-06802) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

11. Blank control (Black line): A431 (Black).

Primary Antibody (green line): Rabbit Anti-EphB2 antibody (TMAB-06802)

Dilution: 1 µg /10⁶ cells;

Isotype Control Antibody (orange line): Rabbit IgG.

Secondary Antibody (white blue line): Goat anti-rabbit IgG-AF647

Dilution: 3 µg /test.

Protocol

The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with 0.1% PBST for 20 min at room temperature. The cells were then incubated in 5% BSA to block non-specific protein-protein interactions for 30 min at room temperature. Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.

12. Blank control: A431.

Primary Antibody (green line): Rabbit Anti-GRP94 antibody (TMAB-06802)

Dilution: 1 µg /10⁶ cells;

Isotype Control Antibody (orange line): Rabbit IgG.

Secondary Antibody: Goat anti-rabbit IgG-AF647

Dilution: 1 µg /test.

Protocol

The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with 0.1% PBST for 20 min at room temperature. The cells were then incubated in 5% BSA to block non-specific protein-protein interactions for 30 min at room temperature. Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.

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Application: WB,IHC-P,IHC-Fr,IF,FCM

Recommended WB: 1:500-2000; IHC-P: 1:100-500; IHC-Fr: 1:100-500; IF: 1:100-500; FCM: 1µg/Test

Properties

Stability & Storage: Store at 2°C-8°C for 1 month. Store at -20°C or -80°C for 12 months. Avoid repeated freeze-thaw cycles.

Shipping: Shipping with blue ice.

Antigen Details

Immunogen: KLH conjugated synthetic peptide: human GRP94

Antigen Species: Human

Gene ID: 7184

Uniprot ID: P14625

Research Background

bs-0194P is one synthetic peptide derived from human GRP94. Glucose regulated protein 94 (GRP 94) is a resident protein of the endoplasmic reticulum (ER) and is induced by the accumulation of unfolded proteins suggesting that it might associate transiently with a variety of newly synthesized secretory and membrane proteins or permanently with mutant or defective proteins. The highly conserved sequence Lys-Asp-Glu-Leu (KDEL) is present at the C terminus of GRP 94 and other resident ER proteins including GRP 78 and protein disulfide isomerase (PDI). The presence of carboxy terminal KDEL appears to be necessary for retention and appears to be sufficient to reduce the secretion of proteins from the ER. This retention is reported to be mediated by a KDEL receptor. GRP 94 is also a low affinity, high capacity calcium binding protein, though its role, if any, in calcium regulation is not understood.

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