For Research Use Only.
Not for use in diagnostic procedures .

Anti-ZBTB24 pAb

CODE No. PM085

CLONALITY Polyclonal
ISOTYPE Guinea Pig Ig, affinity purified
QUANTITY 100 µL

SOURCE Purified Ig from guinea pig serum
IMMUNOGEN Recombinant mouse ZBTB24 (C-terminus)
FORMULATION PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
STORAGE This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS-CONFIRMED

Western blotting 1:1,000
Immunoprecipitation 2 µL/300 µL of cell extract from 1 x 10^6 cells/sample
*Immunoprecipitation for human sample is not recommended.

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	SBC5, MCF7	E14 ES cell	Not tested	Not tested
Reactivity	+	+		

Entrez Gene ID 9841 (Human), 268294 (Mouse)

REFERENCES

1) Nitta, H., *et al., J. Hum. Genet.* **58**, 455-460 (2013)
2) Weemaes, C. M., *et al., Eur. J. Hum. Genet.* **21**, 1219-1225 (2013)
3) Cerbone, M., *et al., Am. J. Med. Genet. A.* **158A**, 2043-2046 (2012)
4) Chouery, E., *et al., Clin. Genet.* **82**, 489-493 (2012)
5) de Greef, J. C., *et al., Am. J. Hum. Genet*. **88**, 796-804 (2011)

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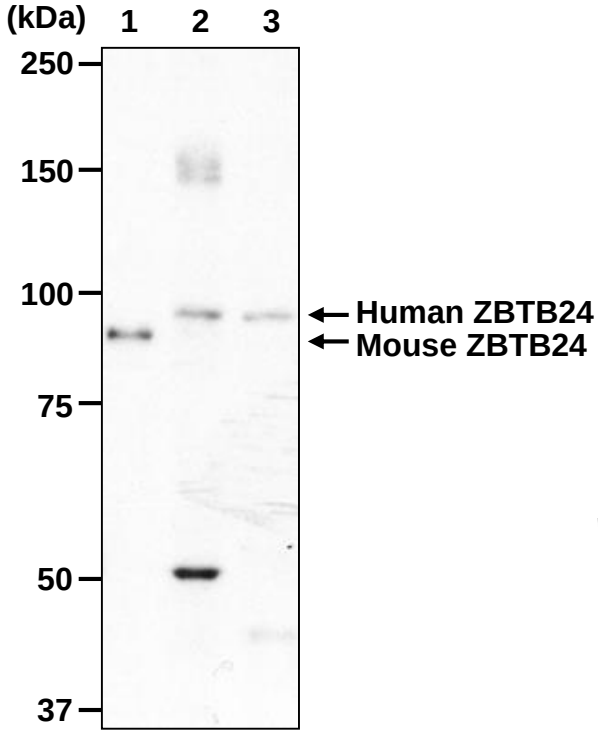
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Wash 5 x 10^6 cells 3 times with PBS and resuspend them in 250 μL of Extraction buffer [20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.5% NP-40], then sonicate for 30 sec.
2) Add equal volume of 2 x Laemmli's sample buffer, then boil for 5 min.
3) Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
5) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T (5 min. x 3 times).
9) Incubate the membrane with 1:20,000 of anti-guniea pig IgG-HRP (Life Technologies; code no. 61-4620) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
10) Wash the membrane with PBS-T (5 min. x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with ECL™ Western Blotting Detection Reagents (GE Healthcare; code no. RPN2106) for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 10 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Mouse E14 ES cell, SBC5 and MCF7)



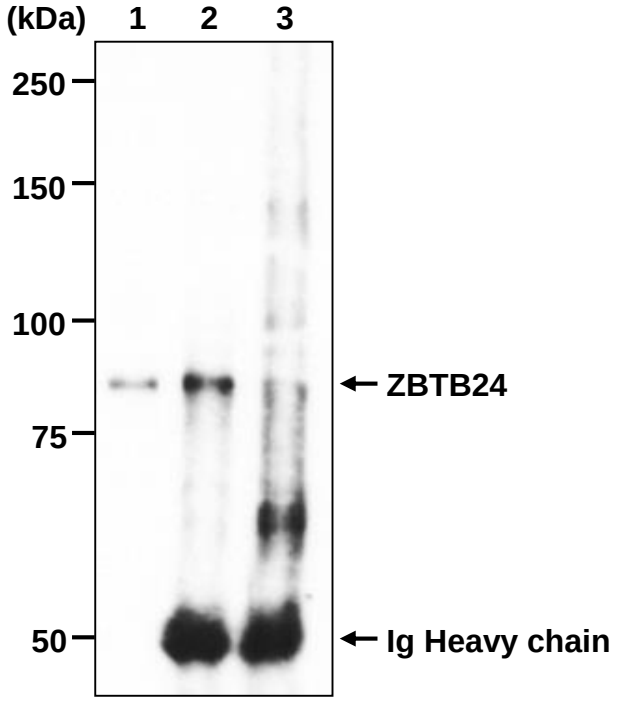
Western blot analysis of ZBTB24
Lane 1: E14 ES cell
Lane 2: SBC5
Lane 3: MCF7

Immunoblotted with Anti-ZBTB24 pAb (PM085)

Immunoprecipitation

1) Wash 3 x 10^6 cells 3 times with PBS and resuspend them in 1 mL of RIPA buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 0.1% SDS) and 10 μL of Protease Inhibitor Cocktail (Sigma; code no. P8340), then sonicate for 30 sec.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Mix 30 μL of 50% protein A agarose beads slurry resuspended in 300 μL of PBS with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at 4°C.
4) Wash the beads 3 times with 1 mL of PBS-T (0.05% Tween-20 in PBS).
5) Add 300 μL of cell lysate (prepared sample from step 2)). Incubate with gentle agitation for 1 hr. at 4°C.
6) Wash the beads 3 times with 1 mL of PBS-T.
7) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
8) Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
9) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
10) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
11) Wash the membrane with PBS-T (5 min. x 3 times).
12) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
13) Wash the membrane with PBS-T (5 min. x 3 times).
14) Incubate the membrane with 1:20,000 of anti-guniea pig IgG-HRP (Life Technologies; code no. 61-4620) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
15) Wash the membrane with PBS-T (5 min. x 3 times)
16) Wipe excess buffer on the membrane, then incubate it with ECL™ Western Blotting Detection Reagents (GE Healthcare; code no. RPN2106) for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
17) Expose to an X-ray film in a dark room for 10 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunopirecipitation; Mouse E14 ES cell)



Immunoprecipitation of ZBTB24 from E14 ES cells
Lane 1: Input (total cell lysate)
Lane 2: Anti-ZBTB24 pAb (PM085)
Lane 3: Normal guinea pig IgG (PM067)

Immunoblotted with Anti-ZBTB24 pAb (PM085)