

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

Anti-ZBTB24 (Human) pAb

CODE No. PM086

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

SOURCE Purified Ig from guinea pig serum
IMMUNOGEN Recombinant human ZBTB24 (C-terminus)
FORMURATION 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 for chemiluminescence detection system
Immunoprecipitation 2 µL/300 µL of cell extract from 1 x 10⁶ cells/sample

SPECIES CROSS REACTIVITY on WB

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

Entrez Gene ID 9841 (Human)

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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RELATED PRODUCTS

Antibodies

PM086	Anti-ZBTB24 (Human) pAb
PM085	Anti-ZBTB24 pAb
D346-3	Anti-5-methylcytidine (m ⁵ C) mAb
M218-3	Anti-5-hydroxymethylcytosine (5hmC) mAb
PM077	Anti-5-hydroxymethylcytosine (5hmC) pAb
M202-3	Anti-Np95 (Uhrf1) mAb (R32-44)
D289-3	Anti-Np95 (Uhrf1) (Mouse) mAb (Th-10a)
PM067	Normal Guinea Pig IgG

Kits

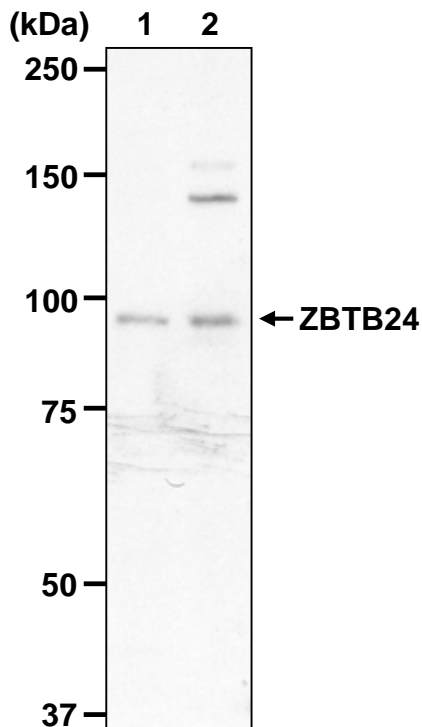
5270-100	MethylHunter MBD1-based Methylated DNA Enrichment Kit
5275-100	MethylHunter MBD1-based Methylated DNA Enrichment Kit 2
5350	MethylHunter 5hmC detection kit

Other related antibodies and kits are also available.
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SDS-PAGE & Western blotting

- 1) Wash 5×10^6 cells 3 times with PBS and resuspend them in 250 μ L 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 μ L 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/cm² 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-guinea 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 ECLTM 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; MCF7 and SBC5)



Western blot analysis of human ZBTB24

Lane 1: MCF7

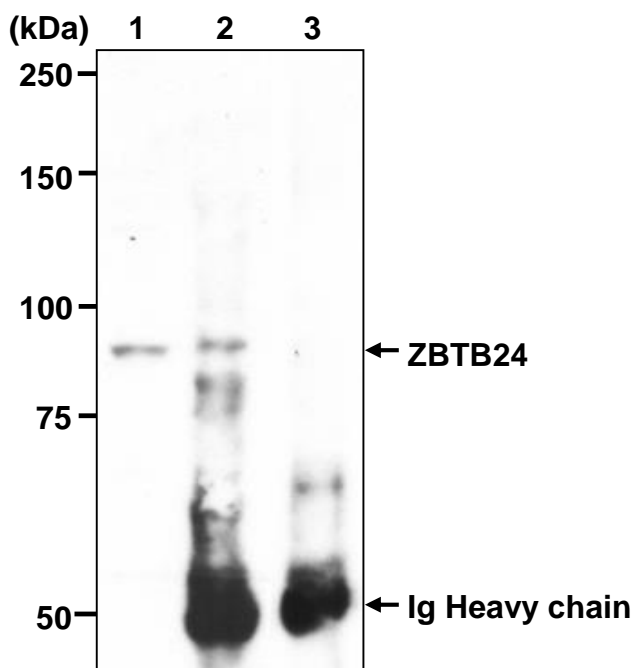
Lane 2: SBC5

Immunoblotted with Anti-ZBTB24 (Human) pAb (PM086)

Immunoprecipitation

- 1) Wash 3×10^6 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 μ L 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 μ L of 50% protein A agarose beads slurry resuspended in 300 μ L 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 μ L 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 μ L of Laemmli's sample buffer, boil for 5 min. and centrifuge.
- 8) Load 10 μ L 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/cm² 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-guinea 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 Immunoprecipitation; MCF7)



Immunoprecipitation of ZBTB24 from MCF7

Lane 1: Input (total cell lysate)
Lane 2: Anti-ZBTB24 (Human) pAb (PM086)
Lane 3: Normal Guinea Pig IgG (PM067)

Immunoblotted with Anti-ZBTB24 (Human) pAb (PM086)