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

POLYCLONAL ANTIBODY

Anti-HP1γ pAb

Code No.	Quantity	Form	Concentration
BMP003	100 µL	Purified IgG	500 µg/mL

SOURCE: This antibody was an affinity chromatography purified rabbit polyclonal antibody raised against synthesized peptide, CSQKAGKEKDGTKRKSLSD, which corresponding to human HP1γ (79-96 aa).

FORMULATION: 50 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human and mouse HP1γ (24 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 0.1-1 µg/mL for chemiluminescence detection system

Immunoprecipitation; 1 µg/ 200 µL of cell extract from $5x10^6$ cells

Immunocytochemistry; 10 µg/mL

Immunohistochemistry; 10 µg/mL
Heat treatment is necessary for paraffin embedded sections.
Microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.5)
This antibody is available for frozen sections in addition to paraffin sections.

Flow Cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:
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SPECIES CROSS REACTIVITY:

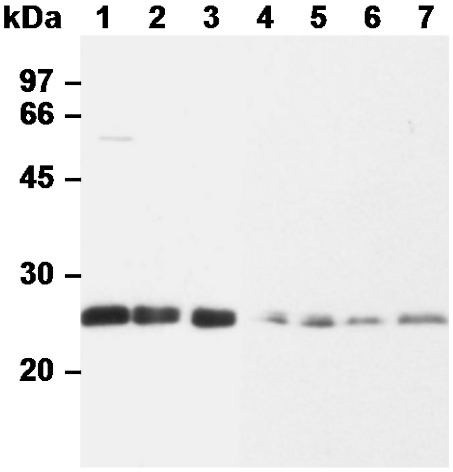
Species	Human	Mouse	Rat	Hamster
Cells	HeLa, Jurkat, Raji	P3U1, WR19L	PC12	CHO
Reactivity on WB	+	+	+	+

REFERENCES:

1) Xin, H., *et al. J. Biol. Chem.* **279**, 9539-9546 (2004)
2) Filesi, I., *et al. J. Cell Sci*. **115**, 1803-1813 (2002)

RELATED PRODUCTS:

BMP001	Anti-HP1α pAb
BMP002	Anti-HP1β pAb
BMP004	Anti-mSIN3A pAb
BMP005	Anti-RbAp48 N-terminal pAb
BMP006	Anti-RbAp48 C-terminal pAb
PM035	Normal Rabbit IgG



Western blot analysis of HP1 γ expression in HeLa cells (1), Jurkat cells (2), Raji cells (3), P3U1 cells (4), WR19L cells (5), PC12 cells (6) and CHO cells (7) using BMP003.

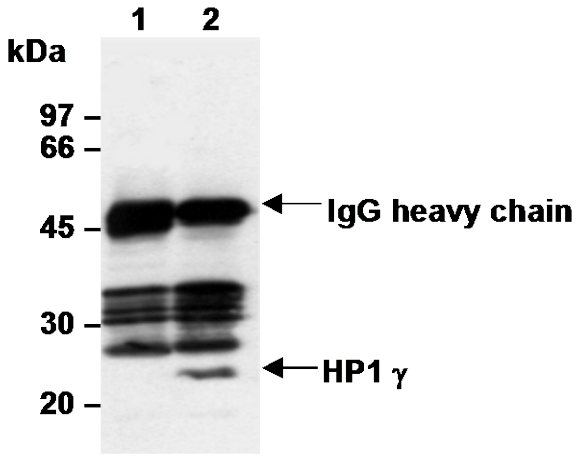
PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the $1x10^7$ cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 20 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (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). See the manufacture's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 5% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend

on condition.)
6) Wash the membrane with PBS (10 minutes x 3 times).
7) Incubate the membrane with 1:10,000 of Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS (10 minutes x 3 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) 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.

(Positive controls for Western blotting; HeLa, Jurkat, Raji, WR19L, P3U1, PC12 and CHO)

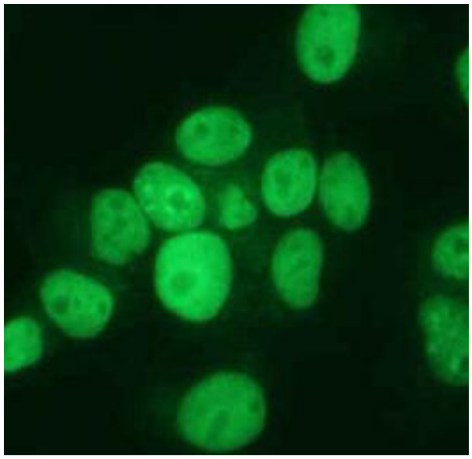


Immunoprecipitation of HP1 γ form HeLa cells with rabbit IgG 1 μg (1) or BMP003 1 μg (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with BMP003.

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM HEPES (pH 7.4), 250 mM NaCl, 0.1% NP-40, 5 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the **APPLICATIONS** into 200 μL of the supernatant. Mix well and incubate with gentle agitation for 60 minutes at room temperature. Add 20 μL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at room temperature.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 μL/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.)

(Positive control for immunoprecipitation; HeLa)



Immunocytochemical detection of HP1 γ on Ethanol fixed HeLa cells with BMP003.

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 10^4 of HeLa cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in 100% ethanol for 30 minutes at 37°C.
4) Immerse the slides in PBS containing 0.05% Tween-20 for 10 minutes at 37°C.
5) Wash the cells 3 times with PBS.
6) Add the primary antibody diluted with PBS as suggest in the **APPLICATIONS** onto the cells and incubate for 30 minutes at 4°C (Optimization of antibody concentration or incubation condition is recommended if necessary.)
7) Prepare a wash container such as a 500 mL beaker with a stirrer. Then wash the cultured cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another wash once more.
8) Add FITC-conjugated anti-rabbit IgG antibody onto the cells. Incubate for 30 minutes at 4°C. Keep out light by aluminum foil.
9) Wash the slide in a plenty of PBS as in the step 6).
10) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
11) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for immunocytochemistry; HeLa)