

POLYCLONAL ANTIBODY

# Anti-HP1 $\gamma$ pAb

Code No.	Quantity	Form	Concentration
BMP003	100 $\mu$ L	Purified IgG	500 $\mu$ g/mL

**SOURCE:** This antibody was an affinity chromatography purified rabbit polyclonal antibody raised against synthesized peptide, CSQKAGKEKDGTKRKSLSD, which corresponding to human HP1 $\gamma$  (79-96 aa).

**FORMULATION:** 50  $\mu$ g IgG in 100  $\mu$ L 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 HP1 $\gamma$  (24 kDa) on Western blotting.

### APPLICATIONS:

Western blotting; 0.1-1  $\mu$ g/mL for chemiluminescence detection system

Immunoprecipitation; 1  $\mu$ g/ 200  $\mu$ L of cell extract from 5x10<sup>6</sup> cells

Immunocytochemistry; 10  $\mu$ g/mL

Immunohistochemistry; 10  $\mu$ g/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:

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

### SPECIES CROSS REACTIVITY:

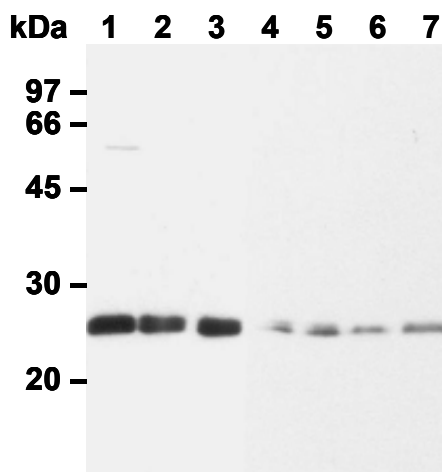
Species	Human	Mouse	Rat	Hamster
Cells	HeLa, Jurkat, Raji	P3U1, WR19L	PC12	CHO
Reactivity on WB	+	+	+	+

### 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-HP1 $\alpha$ pAb
BMP002	Anti-HP1 $\beta$ 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  $\gamma$  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:

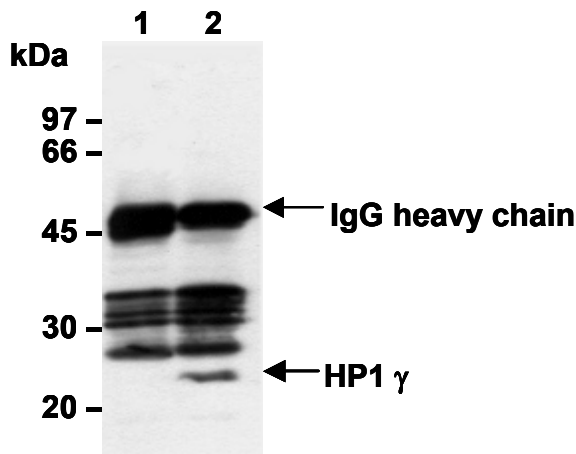
#### SDS-PAGE & Western Blotting

- 1) Wash the 1x10<sup>7</sup> 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  $\mu$ L 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<sup>2</sup> 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)



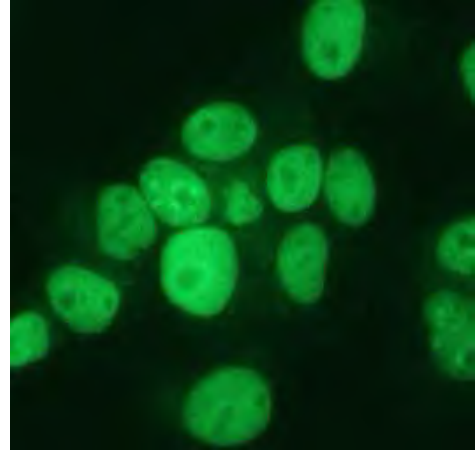
**Immunoprecipitation of HP1  $\gamma$  from HeLa cells with rabbit IgG 1  $\mu$ g (1) or BMP003 1  $\mu$ g (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with BMP003.**

#### **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  $\mu$ L of the supernatant. Mix well and incubate with gentle agitation for 60 minutes at room temperature. Add 20  $\mu$ L 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  $\mu$ L of Laemmli's sample

buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20  $\mu$ L/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.)

(Positive control for immunoprecipitation; HeLa)



**Immunocytochemical detection of HP1  $\gamma$  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<sub>2</sub> 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)