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

POLYCLONAL ANTIBODY

Anti-HP1 α

Code No.	Quantity	Form	Concentration
BMP001	100 µL	Purified IgG	0.5 mg/mL

SOURCE: This antibody was an affinity chromatography purified rabbit polyclonal antibody raised against synthesized peptide, CHAYPEDAENKEKETAKS, which corresponding to human HP1 α (175-191 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 HP1 α (29 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 0.25-1 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 10mM citrate buffer (pH 6.5)
Flow Cytometry; Not tested

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

INTENDED USE:
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SPECIES CROSS REACTIVITY:

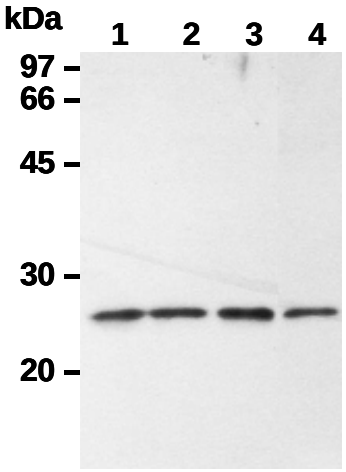
Species	Human	Mouse	Rat
Cell	HeLa, Jurkat, Raji	WR19L	PC12
Reactivity on WB	+	+	–

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:

BMP002	Anti-HP1 β
BMP003	Anti-HP1 γ
BMP004	Anti-mSIN3A
BMP005	Anti-RbAp48 N-terminal
BMP006	Anti-RbAp48 C-terminal



Western blot analysis of HP1 α expression in HeLa cells (1),Jurkat cells (2),Raji cells (3) and WR19L cells (4) using BMP001.

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 to be used will be depend on condition.)

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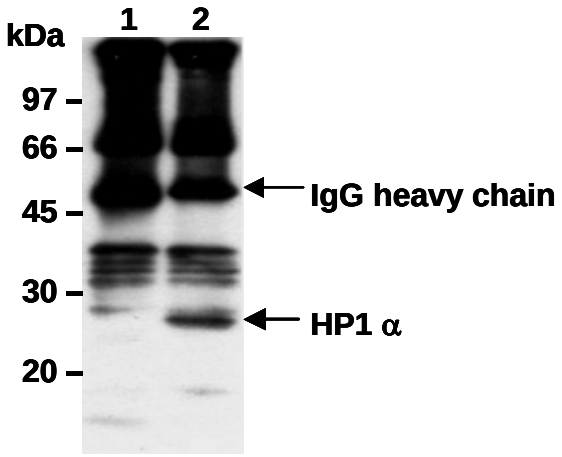
6) Wash the membrane with PBS (10 minutes x 3 times).
7) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rabbit IgG (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS (10 minutes x 3 times).
9) Wipe excess buffer 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)

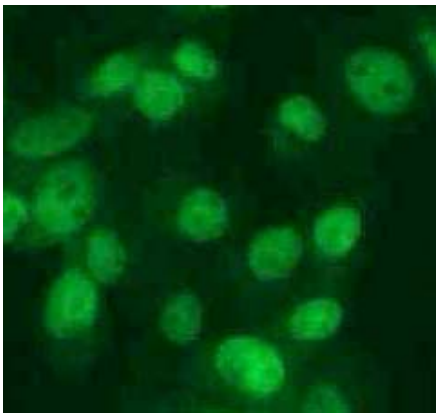


Immunoprecipitation of HP1 α from HeLa cells with rabbit IgG 1 μg (1) or BMP001 1 μg (2).*Affer immunoprecipitated with the antibody,immunocomplex was resolved on SDS-PAGE and immunoblotted with BMP001.*

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 30-120 minutes at 4 °C. 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 4 °C.
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 10 μL/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.)

(Positive control for immunoprecipitation; HeLa)



Immunocytochemical detection of HP1 α on Ethanol fixed HeLa cells with BMP001.

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 room temperature (Optimization of antibody concentration or incubation condition are 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 washes once more.
8) Add the 40 μL of 1:100 FITC conjugated anti-rabbit IgG (MBL code no. IM-0833) onto the cells. Incubate for 30 minutes at room temperature. 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)

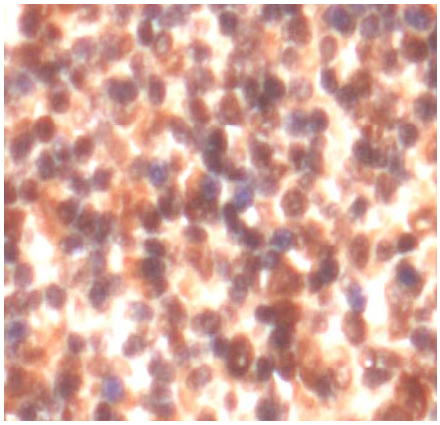
Immunohistochemical staining for paraffin-embedded sections : SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment

Heat treatment by Microwave:
Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.

5) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; MBL, code no. IM-2391) for 5 minutes to block non-specific antibody staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).
12) Visualize by reacting for 1 minutes with substrate solution ENVISION Kit (Dako Japan; Code no. K3467). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting.

(Positive control for immunohistochemistry; Tonsil)



Immunohistochemical detection of HP1 α on paraffin embedded section of Human Tonsil with BMP001.