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MONOCLONAL ANTIBODY

Anti-CENP-A (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D115-3	3-19	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Centromere protein A (CENP-A) is a 17-19 kDa centromere-specific histone variant that is 62% identical to the carboxy-terminal domain of histone H3. In the presence of DNA, CENP-A forms an octameric complex with histones H4, H2A, and H2B. CENP-A defines active centromere regions by forming centromere-specific nucleosomes on which kinetochores are assembled. CENP-A is specifically located to the inner plate of the kinetochore and it is essential for kinetochore targeting of CENP-C and other kinetochore components. CENP-A is required for nucleosomal packaging of centromeric DNA at interphase.

SOURCE: This antibody was purified from hybridoma (clone 3-19) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3-X63-Ag8 with Balb/c mouse splenocyte immunized with human CENP-A peptide (3-19 aa).

FORMULATION: 100 µg IgG in 100 µL volume of 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.

REACTIVITY: This antibody reacts with human CENP-A (17 kDa) on Western blotting, Immunohistochemistry and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not recommended*
*It is reported that this monoclonal antibody can be used in Chromatin Immunoprecipitation (ChIP) in the reference number 9) and 10).
Immunohistochemistry; 5 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)
Immunocytochemistry; 10 µg/mL
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, Raji	WR19L, NIH/3T3	Rat-1, PC12
Reactivity on WB	+	-	-

INTENDED USE:
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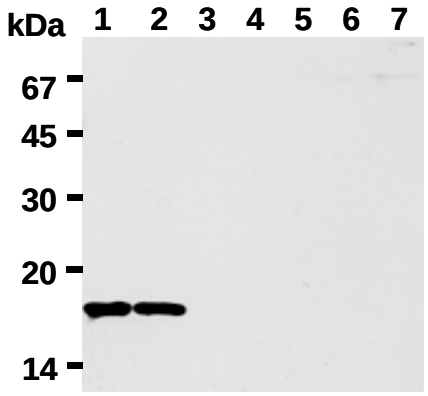
REFERENCES:

1) Kazami, T., *et al.*, *Oncogene* **34**, 4177-4189 (2015) [WB]
2) Okada, M., *et al.*, *Mol. Cell. Biol.* **20**, 3986-3995 (2009) [WB, IC]
3) Marshall, O. J., *J. Cell. Biol.* **183**, 1193-1202 (2008) [IC]
4) Hemmerich, P., *et al.*, *J. Cell Biol.* **180**, 1101-1114 (2008) [IC]
5) Slattery, S. D., *et al.*, *Cell Cycle* **7**, 787-795 (2008) [WB]
6) Chi, Y. H., *et al.*, *J. Biol. Chem.* **282**, 27447-27458 (2007) [IC]
7) Mimori-Kiyosue, Y., *et al.*, *Genes Cells* **11**, 845-857 (2006) [IC]
8) Izuta, H., *et al.*, *Genes Cells* **11**, 673-684 (2006) [IC]
9) Obuse, C., *et al.*, *Genes Cells* **9**, 105-120 (2004) [WB, ChIP]
10) Ando, S., *et al.*, *Mol. Cell. Biol.* **22**, 2229-2241 (2002) [WB, IP, ChIP]



Western blot analysis of CENP-A expression in Jurkat (1), Raji (2) PC12 (3), Rat-1 (4) WR19L (5) C2C12 (6) and NIH/3T3 (7) using D115-3.

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

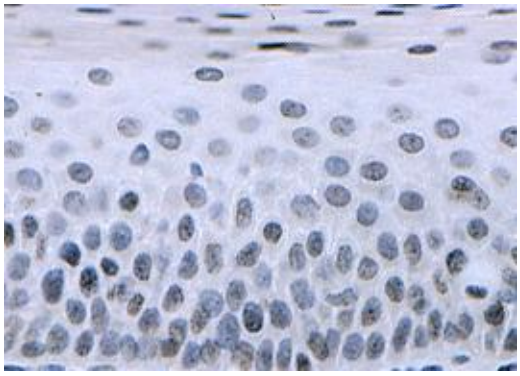
PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2),

250 mM NaCl, 0.1% NP-40, 2 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. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) 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.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurka and Raji)



Immunohistochemical detection of CENP-A on paraffin embedded section of a squamous epithelium of human tonsil with D115-3.

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 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 suggested 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 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *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.

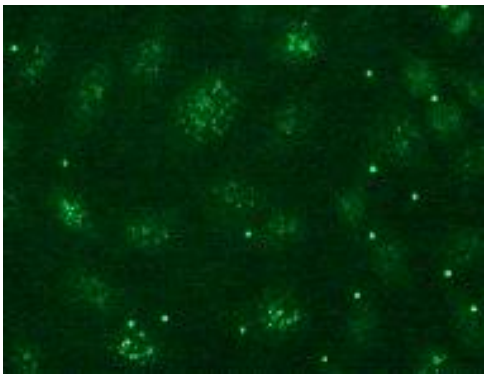
(Positive control for Immunohistochemistry; Tonsil)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1 x 10^4 of HEp-II cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Fix the cells by immersing the slide in Acetone for 10 minutes on ice.
3) Air dry the slides.

4) Add 30 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.09% NaN_3 or Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) on to the cells. Incubate for 10 minutes at room temperature.
   *Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
5) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
6) 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.
7) Add the 30 µL of 1:100 Anti-IgG (Mouse) pAb-FITC (MBL; code no. 238) onto the cells. Incubate for 1 hour at room temperature. Keep out light by aluminum foil.
8) Wash the slide in a plenty of PBS as in the step 6).
9) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
10) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HEp-II)



Immunocytochemical detection of CENP-A on acetone fixed HEp-II with D115-3.

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