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POLYCLONAL ANTIBODY

Anti-CENP-C (Human) pAb

Code No.	Quantity	Form
PD030	100 µL	Guinea pig IgG

BACKGROUND: Centromere protein C (CENP-C/ICEN7) is a 140 kDa protein and a component of the centromere specific chromatin complex called CENP-A chromatin or ICEN[15] in interphase and forms basis for the inner-kinetochore plate in metaphase. CENP-C is essential for the kinetochore function of equal segregation of daughter chromosomes.

SOURCE: This antibody was purified from guinea pig serum using protein A agarose. The guinea pig was immunized with recombinant human CENP-C protein corresponding to N-terminal amino acids (1-426 aa).

FORMULATION: 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-C (140 kDa) on Western blotting, Immunoprecipitation and Immunocytochemistry.

APPLICATIONS:
Western blotting; 1:1,000
Immunoprecipitation; 2-5 µL/300 µL of cell extract from 3 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; 1:1,000

Detailed procedures are provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	HeLa	Not tested	Not tested
Reactivity on IC	+		

INTENDED USE:
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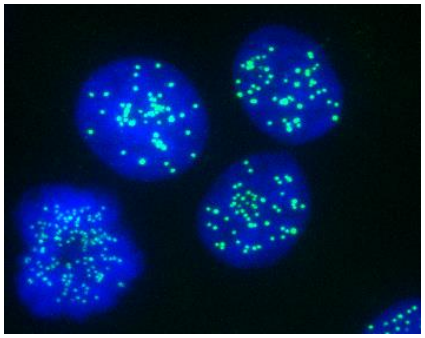
13) Kuijt, T. E., *et al.*, *Chromosoma* **123**, 471-480 (2014) [IC]
14) Ando, S., *et al.*, *Mol. Cell Biol.* **22**, 2229-2241 (2002)
15) Izuta, H., *et al.*, *Genes Cells* **11**, 673-684 (2006)

This antibody is used in the reference number 1)-14).

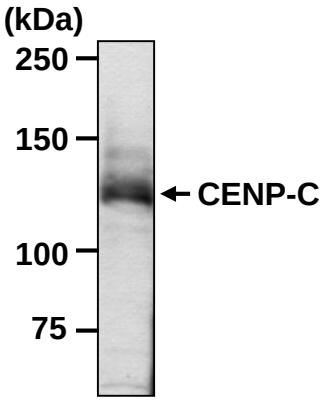


Immunocytochemical detection of CENP-C in HeLa using PD030.
Green: Anti-CENP-C (Human) pAb (PD030)
Blue: DAPI
This data was provided by Dr. Tatsuo Fukagawa. (Department of Molecular Genetics, National Institute of Genetics and The Graduate University for Advanced Studies)

PROTOCOLS:
Immunocytochemistry
1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 10^4 of cells per one well, then incubate in a CO_2 incubator overnight.)
2) Fix the cells by immersing the slide in Methanol for 20 minutes at -20°C.
3) Immerse the slide in 0.5% BSA in PBS for 15 minutes at room temperature.

4) Add the primary antibody diluted with 0.5% BSA in PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at 37°C. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
5) Wash the slide with 0.5% BSA in PBS 3 times for 5 minutes.
6) Add 1:1,000 FITC conjugated anti-Guinea pig IgG (Jackson ImmunoResearch, code no. 106-095-003) diluted with 0.5% BSA in PBS onto the cells. Incubate for 45 minutes at 37°C. Keep out light by aluminum foil.
7) Wash the slide with 0.5% BSA in PBS 3 times for 5 minutes.
8) Counterstain with DAPI for 5 minutes at room temperature.
9) Wash the cells with PBS (5 minutes x 3).
10) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry. Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa)

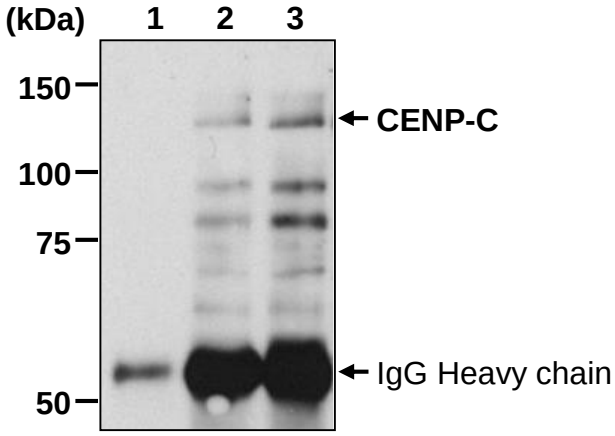


Western blot analysis of CENP-C in HeLa nuclear extract using PD030.

SDS-PAGE & Western blotting

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer [250 mM sucrose, 5 mM Tris-HCl (pH 7.5)] containing protease inhibitors at appropriate concentrations then briefly sonicate the mixture (up to 10 seconds).
2) Centrifuge the tube at 600 x g for 10 minutes at 4°C and discard the supernatant to another tube.
3) Resuspend the pellet (nuclear fraction) with 200 µL 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 and carry out 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% methanol). See the manufacturer'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 for 1 hour at room temperature with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
9) Incubate the membrane with 1:20,000 HRP-conjugated anti-Guinea pig IgG (Thermo Fisher Scientific, code no. 61-4620) 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).
11) Wipe excess buffer off the membrane, and incubate membrane with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
13) Expose the membrane onto an X-ray film in a dark room for 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive control for Western blotting; HeLa nuclear extract)



Immunoprecipitation of CENP-C from YT using PD030. *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PD030.*
Lane 1: Normal Guinea pig IgG
Lane 2: Anti-CENP-C (Human) pAb (PD030), 2 µL
Lane 3: Anti-CENP-C (Human) pAb (PD030), 5 µL

Immunoprecipitation

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 150 mM NaCl, 0.05% NP-40] containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (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 fresh tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 60-120 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) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
5) Resuspend the agarose with cold Lysis buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Repeat steps 5)-6) 2-4 times.
8) 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; YT)

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