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Not for use in diagnostic procedures.

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

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2) Liang, C., *et al.*, *J. Biol. Chem.* **294**, 1437-1450 (2018) [IC]
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11) Etemed, B., *et al.*, *Nat. Commun.* **6**, 8987 (2015) [IC]
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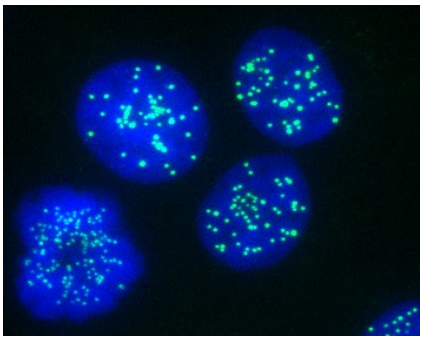
15) Izuta, H., *et al., Genes Cells* **11**, 673-684 (2006)

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)

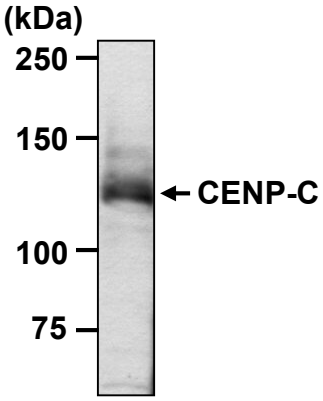
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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)

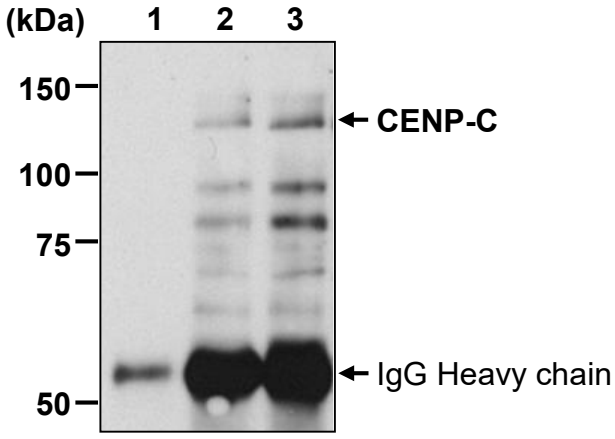


Western blot analysis of CENP-C in HeLa nuclear extract using PD030.

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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