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

MONOCLONAL ANTIBODY

Anti-DNA Polymerase α mAb

Code No.	Clone	Subclass	Quantity	Concentration
MT-20-3	CL22-2-42B	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: DNA polymerases are essential enzymes involved in DNA replication and in repair. The human genome contains 16 distinct known DNA polymerase genes, and these are classified into four families A, B, X, and Y based on their amino acid sequences. DNA polymerases α (pol α) belong to class B DNA polymerases. The pol α is the only known DNA polymerase able to initiate *de novo* DNA synthesis and is required for both initiation at chromosomal origins and for the synthesis of Okasaki fragments on the lagging strand. The pol α is composed of four subunits (p180, p68, p58, and p48) with distinctive functions. The polymerase activity is associated with the largest (p180) subunit, whereas the primase activity is associated with the p48 subunit. The p58 subunit contains a nuclear localization signal that is capable of directing both the p58 monomer and the p48-p58 dimer to the nucleus. The p68 subunit binds tightly to the p180 subunit. The p68 subunit is essential for initial DNA synthesis, and is phosphorylated and dephosphorylated in a cell cycle-dependent manner.

SOURCE: This antibody was purified from hybridoma (clone CL22-2-42B) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell NS-1 with F1 mouse (C57BL6 x Balb/c) splenocyte immunized with the purified DNA polymerase α from calf thymus.

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 DNA polymerase α on Immunocytochemistry and Flow cytometry.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Jurkat	Not tested	
Reactivity on FCM	+		-

INTENDED USE:
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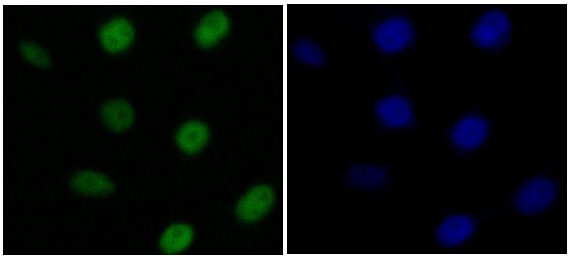
APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry (for cryostat section); 1-2.5 µg/mL
Immunocytochemistry; 2 µg/mL
Flow cytometry; 2 µg/mL (final concentration)

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

REFERENCES:
1) Samudio, I., *et al*., *Endocrinology* **142**, 1000-1008 (2001)
2) Lattanzi, G., *et al*., *J. Histochem. Cytochem.* **46**, 1435-1442 (1998) [IHC]
3) Yamaguchi, A., *et al*., *Br. J. Cancer* **65**, 421-4 (1990) [IHC]
4) Yamaguchi, A., *et al*., *Br. J. Cancer* **61**, 390-3 (1990) [IHC]
5) Mushika, M., *et al*., *Cancer* **61**, 1182-1186 (1988)
6) Masaki, S., *et al*., *Nucleic Acids Research* **12**, 4455-4467 (1984)
7) Nakamura, H., *et al*., *Exp. Cell Res.* **151**, 123-133 (1984)
8) Masaki, S., *et al*., *Nucleic Acids Research* **10**, 4703-4713 (1982)

Clone CL22-2-42B is used in the reference number 1) - 4).



Immunocytochemical detection of DNA polymerase α on paraformaldehyde fixed HeLa with MT-20-3.
Green: Anti-DNA polymerase α mAb
Blue: DAPI counterstain

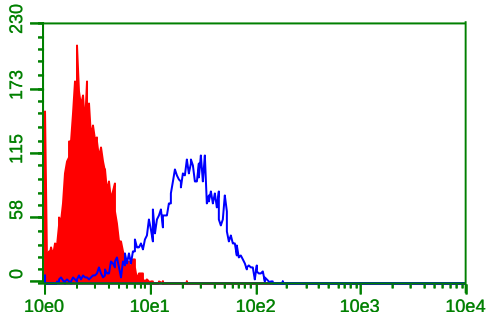
PROTOCOLS:

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass chamber slide. (for example, spread $1x10^5$ of cells per one well, then incubate in a CO_2 incubator for one night.)
2) Wash the slide 2 times with PBS.
3) Fix the cells by immersing the slide in 4%

paraformaldehyde (PFA) in PBS for 10 minutes at room temperature (20~25°C).
4) Wash the slide 2 times with PBS.
5) Permeabilize the cells with 0.1% Triton X-100 in PBS for 10 minutes at room temperature.
6) Wash the slide 2 times with PBS.
7) Block the cells with Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) for 5 minutes at room temperature
8) Incubate the cells with the primary antibody diluted with PBS as suggested in the **APPLICATIONS** for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide 3 times with PBS.
10) Incubate the cells with 1:400 of Alexa Fluor® 488 Goat Anti-mouse IgG (Thermo Fisher Scientific; code no. A11001) diluted with PBS for 30 minutes at room temperature. Keep out light by aluminum foil.
11) Wash the slide 3 times with PBS.
12) Counterstain with DAPI.
13) Wipe excess liquid from 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)



Flow cytometric analysis of DNA polymerase α expression in Jurkat cells. *Open histogram indicates the reaction of MT-20-3 to the cells. Shaded histogram indicates the reaction of isotypic control to the cells.*

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3]. *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.
2) Add 1 mL of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 10 minutes at 4°C.
3) Wash the cells 3 times with 5 mL of washing buffer.
4) Add 1 mL of PBS containing 0.1% TritonX-100 to the cell pellet after tapping. Mix well, then permeablize the cells for 10 minutes at 4°C.
5) Wash the cells 3 times with 5 mL of washing buffer.
6) Dispense the cell suspension to each tube ($5x10^5$ cells/tube). Centrifuge at 3,000 x for 1 minute and carefully remove the supernatant.
7) Add 10 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature (20~25°C).
8) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 15 minutes at room temperature.
9) Wash the cells 1 time with 1 mL of washing buffer.
10) Add FITC conjugated anti-mouse IgG antibody diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
11) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
12) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Jurkat)

RELATED PRODUCTS:

K0160-3	Anti-DNA polymerase δ p125 Catalytic Subunit mAb (5G1)
K0161-3	Anti-DNA polymerase δ p50 Catalytic Subunit mAb (7B4)
M075-3	Mouse IgG1 (isotype control) (2E12)