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Product datasheet for AM26670PU-S

DNA polymerase alpha (POLA1) Mouse Monoclonal Antibody [Clone ID: CL22-2-42B]

Product data:

Product Type:	Primary Antibodies
Clone Name:	CL22-2-42B
Applications:	FC, IF, IHC
Recommended Dilution:	Immunoghistochemistry on frozen sections: 1-2.5 µg/ml. Immunocytochemistry: 1-2.5 µg/ml. Flow cytometry: 2 µg/ml (final concentration). For details see protocols below.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Purified DNA polymerase alpha from calf thymus
Specificity:	This antibody reacts with DNA polymerase alpha.
Formulation:	PBS (pH 7.2) with 1 % sucrose State: Purified State: Lyophilized Ig fraction Preservative: 0.09 % NaN_3
Reconstitution Method:	Dissolve in 100 µl distilled water.
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Prior to reconstitution store at 2-8°C. Following reconstitution store undiluted at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	polymerase (DNA) alpha 1, catalytic subunit
Database Link:	Entrez Gene 5422 Human P09884

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Background: Plays an essential role in the initiation of DNA replication. During the S phase of the cell cycle, the DNA polymerase alpha complex (composed of a catalytic subunit POLA1/p180, a regulatory subunit POLA2/p70 and two primase subunits PRIM1/p49 and PRIM2/p58) is recruited to DNA at the replicative forks via direct interactions with MCM10 and WDHD1. The primase subunit of the polymerase alpha complex initiates DNA synthesis by oligomerising short RNA primers on both leading and lagging strands. These primers are initially extended by the polymerase alpha catalytic subunit and subsequently transferred to polymerase delta and polymerase epsilon for processive synthesis on the lagging and leading strand, respectively. The reason this transfer occurs is because the polymerase alpha has limited processivity and lacks intrinsic 3' exonuclease activity for proofreading error, and therefore is not well suited for replicating long complexes.

Synonyms: DNA polymerase alpha catalytic subunit

Note: This product was originally produced by MBL International.

Protocol: Immunocytochemistry
1) Culture the cells in the appropriate condition on a glass sl ide. (for example, spread 10 4 of cells per one well, 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 the primary antibody diluted with PBS as suggest ed in the A PPLICATIONS onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition are recommended if necessary.)
5) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the c ultured 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.
6) Add 3 0 µ L of 1: 40 FITC conjugated anti - mouse IgG diluted with PBS onto the cells. Incubate for 30 mi n utes at room temperature. Keep out light by aluminum foil.
7) Wash the slide in a plenty of PBS as in the step 5).
8) 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; HEp - II)

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 0.1% NaN 3].
2) Add 200 µ L of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 15 minutes at 4 o C .
3) Wash the cells 3 t imes with washing buffer.
4) Add 200 µ L of PBS containing 0.1% TritonX - 100 to the cell pellet after tapping. Mix well, then permeablize the cells for 10 minutes at 4 o C .
5) Wash the cells 3 times with washing buffer.

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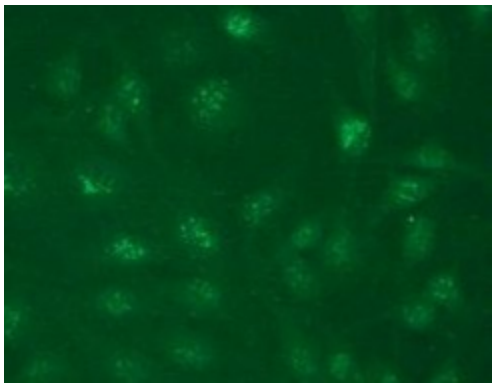
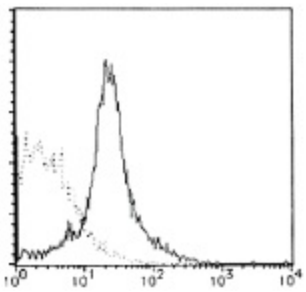
6) Add 2 0 µ L of normal goat serum containing 1 mg /mL normal human IgG and 0.1% NaN 3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature (20~25 o C).
7) Add 40 µ L of the primary antibody at the concentration as suggested in the APPLICATIONS diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
8) Add 1 m L of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 40 µ L of 1:100 FITC conjugated anti - mouse IgG diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
10) Add 1 m L of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
11) Resuspend the cells with 500 µ L of the washing buffer and analyze by a flow cytometer. (Positive control for Flow cytometry; Jurkat)

Product images:



Immunocytochemical detection of DNA polymerase alpha on aceton fixed HEp-II with AM26670PU-S



Flow cytometric analysis of DNA polymerase alpha expression in Jurkat cells. Dot line histogram indicates the reaction of isotypic control to the cells. Solid line histogram indicates the reaction of AM26670PU-S to the cells.

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