Anti-human DNA polymerase δ catalytic subunit/p125 (PolD1) antibody, mouse monoclonal (8A5E3)

Product code	70-051
Size	50 µg
Storage	-20℃
Concentration	1.0 mg/ml
Buffer	PBS- with 50% glycerol
Purity	Purified IgG fraction with protein A from hybridoma cell culture medium
Immunogen	Purified recombinant human PolD1 polypeptide from 247 to 1107aa
Isotype	mouse IgG2bκ (Some contamination of IgM signal)
Reactivity	human PolD1 protein. Other species have not been tested.
Special notes	Specificity has been validated by western blotting with immunoprecipitated samples (Fig. 2)
Application	1. Western blotting (0.33-1 µg/ml) Fig.1 2. Immunoprecipitation (2-6 µg/µl Ab capture beads). This antibody can precipitate Polδ complex from human 293T cell lysate (Fig. 2).
Background	DNA polymerase δ (Pol δ) is one of the eukaryotic B-family polymerases and involved in DNA repair and chromosomal DNA replication. It is composed of a large catalytic subunit encoded by *POLD1* and three accessory subunits; *POLD2*, *POLD3*, and *POLD4*, which encode proteins p125, p50, p66, and p12, respectively. The 125kDa catalytic subunit possesses both polymerase and 3' to 5' exonuclease activity and is able to synthesize DNA strand with high fidelity in the presence of its processivity factor PCNA (proliferating cell nuclear antigen). In the replication fork, starting with the low fidelity Pol α synthesizing a ~30 nt RNA/DNA initiator primer, Pol δ synthesizes a major part of 100 ~200 nucleotide (nt) length-lagging strands, Okazaki fragments, discontinuously, which are then ligated to form the contiguous lagging strand. For synthesis of each Okazaki fragment, Replication factor C (RFC) loads PCNA at the primer/template (P/T) junction. PCNA encircles the duplex DNA and tethers Pol δ complex to the P/T junction. Mutations in this gene have been associated with various cancers and immunodeficiency in human cells.
Data Link	UniProt P28340 (DPOD1_HUMAN)

Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR MILITARY USE.

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Data Images: 70-051 Anti-human DNA polymerase δ catalytic subunit/p125 (PolD1) antibody, mouse monoclonal (8A5E3)

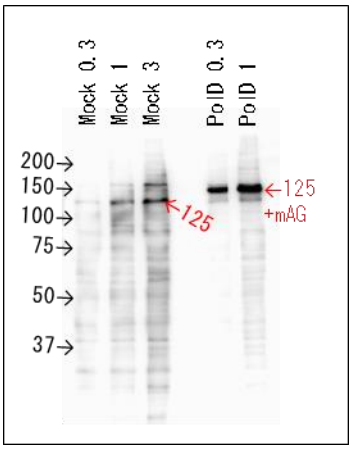

Fig 1. Western blotting of 293T cell lysates with 8A5E3

Indicated amounts (µl) of 293T cell lysate (mock; 13µg protein/µl) or 293T cell lysate expressing PolD1, 2, 3 and 4 subunits (PolD; 9.8µg protein/µl) were electrophoresed in a 12.5% PAAG and transferred to a nylon filter with a semidry blotter. Note that this expressing PolD1 protein has hmAzami-Green tag (mAG) and appears about a 150kDa peptide. This filter was masked with 5% skim milk and the p125 peptide was detected with 0.33µg/ml 8A5E3 in CANGET signal Sol.1, 0.2 µg/ml anti-mIgG-H+L-HRP (ab205719) in CANGET signal Sol.2 and ImmunoSTAR zeta

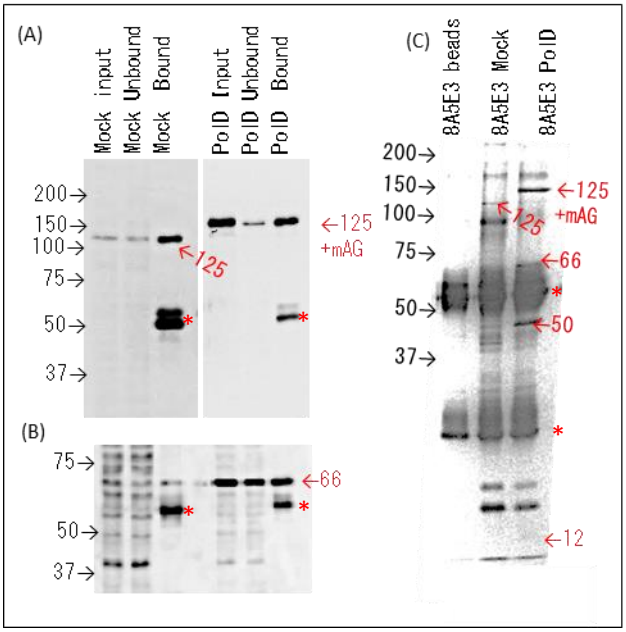

Fig. 2 Immunoprecipitation of Pol δ complex with 8A5E3.

5µl Ab-cap beads were bound with 8A5E3 30 µg. 2 µl each of the beads was mixed with 0.65mg of 295T lysate (Mock) or 0.2mg of 293T lysate expressed with mAG-Polδ complex (PolD), respectively. After washing with PBS containing 10% glycerol, 1mM EDTA and 0.1%NP40, the beads were suspended with 20µl of SDS sample buffer. (A) 3 or 1µl of the samples (input, unbound, and bound)

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were electrophoresed and blotted to nylon filter. Mock input (39µg) and PolD input (9.8µg) were used. PolDp125 peptide was detected with 0.33 µg/ml 8A5E3 in CANGET signal Sol.1, 0.2 µg/ml anti-mIgG-H+L-HRP (ab205719) in CANGET signal Sol.2 and ImmunoSTAR zeta. (B) PolDp66 was detected by western blotting with anti-human PolDp66 monoclonal antibody (2A1C11, #70-052) in the same precipitates as (A). (C) 3µl of the bound samples in the same experiment were electrophoresed and stained with silver. Bands with asterisks are mouse IgG heavy or light chains.

References : This product came from references 3

1. Hindges R and Hubscher U "DNA polymerase delta, an essential enzyme for DNA transactions" *Biol Chem* **378**: 345-362 (1997) PMID: 9191022
2. Johnson A and O'Donnell M "Cellular DNA replicases: components and dynamics at the replication fork" *Annu Rev Biochem* **74**: 283-315 (2005) PMID: 15952889
3. Shikata K *et al* "The human homologue of fission Yeast cdc27, p66, is a component of active human DNA polymerase delta" *J Biochem* **129**: 699-708 (200)

Related products

70-052 Anti-human DNA polymerase δ accessory subunit/p66 (PolD3) antibody, mouse monoclonal (2A1C11)

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