

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

Product code	70-052
Size	50 μ g
Storage	-20°C
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 full-length PolD3 protein
Isotype	mouse IgG2b κ
Reactivity	human PolD3 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 μ g/ml) Fig.1 2. Immunoprecipitation (2-6 μ g/ μ l Ab capture beads). This antibody can precipitate Pol δ 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 <i>POLD1</i> and three accessory subunits; <i>POLD2</i> , <i>POLD3</i> , and <i>POLD4</i> , 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. p66 plays a role in regulating the activity of Pol δ through interactions with other subunits and PCNA.
Data Link	UniProtKB/Swiss-Prot Q15054 (DPOD3_HUMAN)
Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR MILITARY USE.	

Data Images: 70-052 Anti-human DNA polymerase δ accessory subunit/p66 (PoID3) antibody, mouse monoclonal (2A1C11)

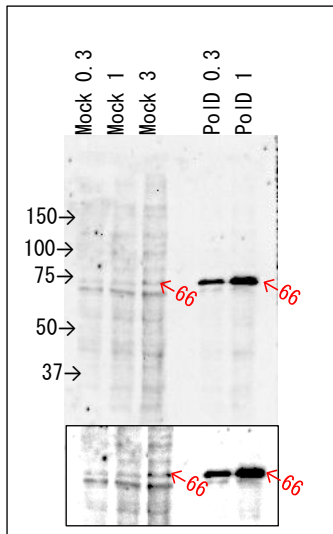


Fig 1. Western blotting of 293T cell lysates with 2A1C11

Indicated amounts (μ l) of 293T cell lysate (Mock: 13 μ g protein/ μ l) or 293T cell lysate expressing PoID1, 2, 3 and 4 subunits (PoID; 9.8 μ g protein/ μ l) were electrophoresed in a 12.5% PAAG and transferred to a nylon filter with a semidry blotter. This filter was masked with 5% skim milk and the p66 peptide was detected with 0.33 μ g/ml 2A1C11 in CANGET signal Sol.1, 0.2 μ g/ml anti-mIgG-H+L-HRP (ab205719) in CANGET signal Sol.2 and ImmunoSTAR zeta. Inserted box is the longer exposure of the 50-100kDa area.

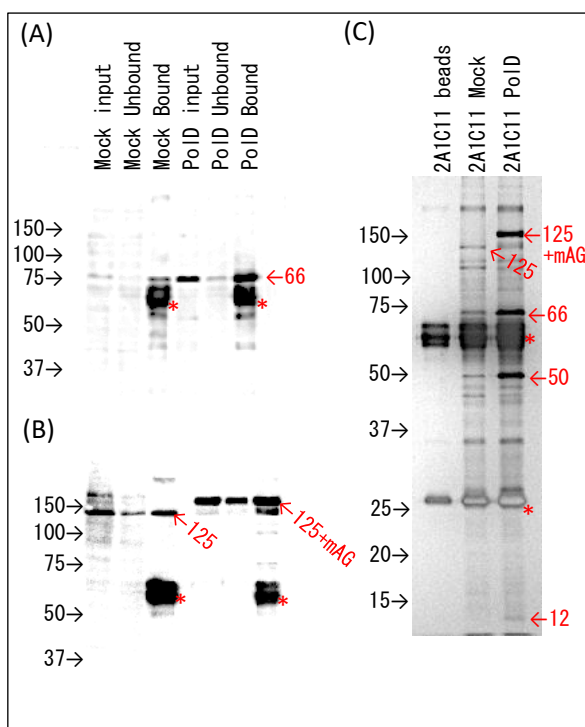


Fig. 2 Immunoprecipitation of Pol δ complex with 2A1C11.

10 μ l of Ab-cap beads were prebound with 30 μ g of 2A1C11. 4 μ l each of the beads was mixed with 0.65mg of 295T mock lysate (Mock) or 0.2mg of 293T lysate expressed with mAG-PolD complex (PolD), respectively.

Note that this expressing PolD1 protein has hmAzami-Green tag (mAG) and appears about a 150kDa peptide. After washing with PBS containing 10% glycerol, 1mM EDTA and 0.1%NP40, the beads were suspended with 20 μ l of SDS sample buffer.

3 μ l (Mock) or 1 μ l (PolD) of the samples (input, unbound, and bound) were electrophoresed in 12.5% PAAG and blotted to nylon filter. Mock input (39 μ g) and PolD input (9.8 μ g) were used. (A) PolDp66 peptide was detected with 0.33 μ g/ml 2A1C11 in CANGET signal Sol.1. (B) PolDp125 peptide or 150kDa peptide of p125 with mAG-tag were detected with 0.33 μ g/ml 8A5E3 (E70-051) in CANGET signal Sol.1. Both antibodies were detected with 0.2 μ g/ml anti-mIgG-H+L-HRP (ab205719) in CANGET signal Sol.2 and ImmunoSTAR Z. (C) 3 μ l of the bound samples (Mock and PolD) of the immunoprecipitation were electrophoresed with the beads-bound antibody (Blank), 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:

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

70-053 Anti-human DNA polymerase δ accessory subunit/p66(PolD3) recombinant antibody with mouse Fc (r2A1C11LvR31)