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

Product code	70-053
Size	50 µg
Storage	-20℃
Concentration	1.0 mg/ml
Buffer	PBS- with 40% glycerol
Preparation scheme and purity	This recombinant antibody was expressed from human 293T cells transfected with the expression plasmid. The culture sup was successively applied to Ab-capture beads and Ni-NTA agarose, and the final product of about 90% pure was obtained. The rest, 10% was the degraded product.
RNA Source	A hybridoma cell line (2A1C11) obtained from mice immunized with full-length PolD3 protein.
Structure	A 58kDa protein, carrying 123aa. of variable heavy chain and 120aa. of variable light chain followed by 231aa. mouse IgG2a Fc region with 6xHis tag at the C-terminal.
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 (0.33-1µg/ml) Fig.1. Recommend to use anti-mIgG-FC-HRP (ab97264) as the second antibody. 2. Immunoprecipitation (2-6µg/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. 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 (proliferating cell nuclear antigen) 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)

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

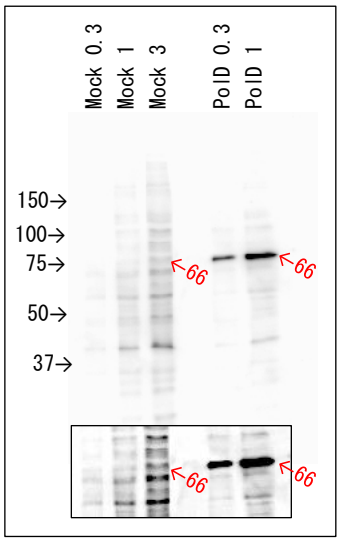

Fig 1. Western blotting of 293T cell lysates with r2A1C11LvR31

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. This filter was masked with 5% skim milk and the p66 peptide was detected with 0.33 µg/ml r2A1C11LvR31 in CANGET signal Sol.1, 0.2µg/ml anti-mIgG-FC-HRP (ab97264) in CANGET signal Sol.2 and ImmunoSTAR zeta. Inserted box is the longer exposure of the 50-100kDa area.

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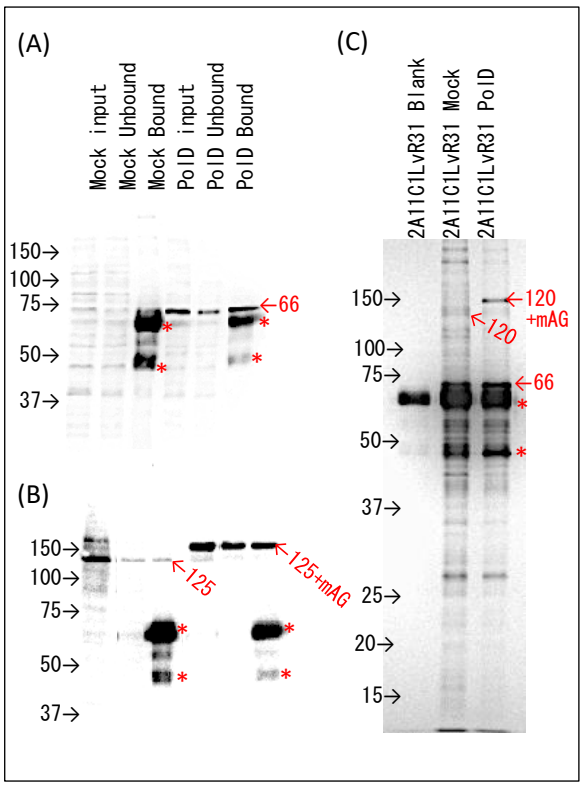

Fig. 2　Immunoprecipitation of Pol δ complex with r2A1C11LvR31.

10µl of Ab-cap beads were prebound with 30µg of r2A1C11LvR31. 4µl 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 20µl of SDS sample buffer.

3µl (Mock) or 1µl (PolD) of the samples (input, unbound, and bound) were electrophoresed in 12.5% PAAG and blotted to nylon filter. Mock input (39µg) and PolD input (9.8µg) were used.

(A) PolDp66 peptide was detected with 0.4µg/ml r2A1C11LvR31 in CANGET signal Sol.1. (B) PolDp125 peptide or about 150kDa peptide of p125 with mAG-tag were detected with 0.33µg/ml 8A5E3 (70-051) in CANGET signal Sol.1. Antibodies were detected with 0.2µg/ml of anti-mIgG-FC-HRP (For (A); ab97264) or anti-mIgG-H+L-HRP (For (B); ab205719) in CANGET signal Sol.2 and ImmunoSTAR Z. (C) 3µl 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 the recombinant antibody fragment and its degraded produce.

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

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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)

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

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