Anti-DNA ligase1 (human) antibody, rabbit polyclonal

70-090　　100 μg

Shipping and Storage: Shipped at 4℃ or -20℃ and store at -20℃. Do not freeze.

Immunogen: Recombinant FLAG-Lig1 highly purified from insect cells infected with baculovirus designed for expression of the full-length FLAG-Lig1.

Form: 1mg/ml in PBS- with 50% glycerol. Filter-sterilized.

Purity: Protein A purified IgG

Reactivity: It is able to detect a specific 140kDa band with human cell lysates by immunoblotting (Fig. 1), slightly shifted up from its calculated MW (100kDa).

Applications:

1.Western blotting: 1 μg/ml (Fig.1)

2.Immunofluorescent staining: 0.5 μg/ml (Fig.2)

Background: *LIG1* (HGNC: 6598) encodes one of the ATP-dependent DNA ligases in human cells, which functions in DNA replication, recombination, and the base excision repair process. Mutations in this gene lead to DNA ligase I deficiency and result in immunodeficiency and increased sensitivity to DNA-damaging agents, suggesting its relation to cancer development. Human cells have two additional DNA ligase families, LIG3 and LIG4, both of which have redundant roles to LIG1 in DNA replication and DNA damage responses and their mutations are also involved in cancer development (Annu Rev Biochem. 2008; 77: 313–338). Several Lig1-interacting proteins have been identified. One of the interacting proteins is DNA-sliding clamp, the homotrimeric proliferating cell nuclear antigen (PCNA), which are involved in DNA replication and repair. The interaction with PCNA is primarily mediated by a conserved PCNA-binding motif, PIP box, at the N terminus of Lig1. The interaction with PCNA is required for localization of Lig1 at the sites of DNA replication, the Okazaki fragment joining and the base excision repair.

Data Link: UniProtKB - P18858 (DNLI1_HUMAN)

Reference:

1. Patrick Maffucci, *et al.* Biallelic mutations in DNA ligase 1 underlie a spectrum of immune deficiencies. J.Clin Invest. 128(12). :5489-5504 (2018) PMID: 30395541. **WB**

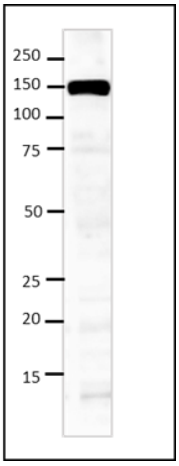

Fig. 1 Detection of endogenous Lig1 in whole cell extract of human HEK293T cells

34 µg of the HEK293T cell lysate was separated on 10% SDS-PAGE and blotted on PVDF membrane with a wet-transfer apparatus at 15V overnight. After blocking of the membrane with 5% skim milk for 95min, anti-Lig1 antibody was applied to it at 1µg/ml for 145min, and then, Goat Anti-Rabbit IgG H&L (HRP) (ab205718) was applied at 1/10,000 dilution at RT for 90min. The reacted antibody was detected with Immunostar zeta reagent (291-72401, wako). A 140kDa band corresponding to the cellular Lig1 could be detected.

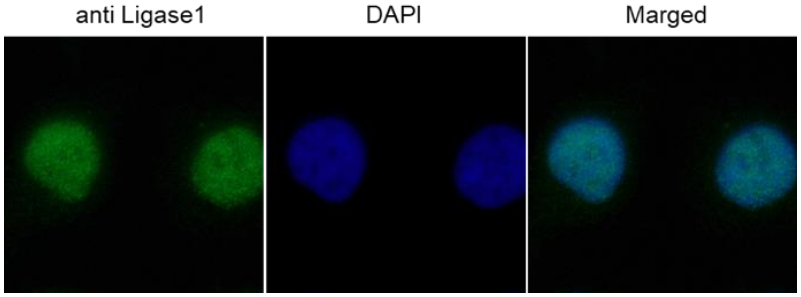

Fig. 2 Immunofluorescence detection of HeLa cell nuclei

Hela cell grown in a dish was fixed with 4 % PFA at 4℃ for 30min. and permeabilized with 0.25 % Triton X-100 in PBS- for 10 min. 0.5µg/ml of anti-RPA antibody was applied to the sample at 4℃ overnight after blocking with 2% BSA in PSB- at RT for 75min. The reacted antibody was detected with Goat anti rabbit IgG-H&L (Alexa Fluor 488) (ab150077) at 1/1000 dilution for 70min. DAPI staining was done with 1.0µg/mL DAPI in TBS for 5 min. Fluorescent signals were observed by using Ts2-FL (Nicon). Fluorescent images were analyzed in NIS-Elements D (Nicon).

The nuclei were stained specifically with this antibody.

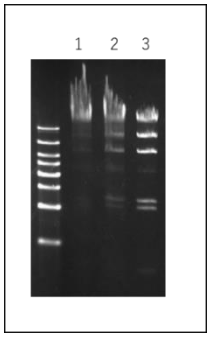

Fig.3 Inhibition of ligase activity by anti Lig-1 antibody

1. λ Hind III digest + Ligase-1
2. λ Hind III digest + Ligase-1 + + anti Lig-1 antibody
3. λ Hind III digest

protocol:

Ligase-1 + anti Lig-1 antibody reaction on ice for 30min

add λ Hind III digest at 16℃ for 30min