Human DNA Ligase 1 tagged with DYKDDDDK-peptide (tag-hLig1), Functional

Product code	10-141
Size	50 µg
Storage	-20℃
Concentration	1 mg/ml
Buffer	25 mM Hepes pH8.0, 1 mM EDTA, 0.01 % NP40, 0.1 mM PMSF, 2 µg/ml leupeptin, 1mM DTT, 0.25M NaCl and 40% glycerol. (No preservative nor carrier protein)
Preparation scheme and purity	Human 293T cell was transfected with plasmid DNA arranged to express full-length human Lig1 (hLig1) tagged with DYKDDDDK-peptide (tag-hLig1). The highly purified tag-hLig1 (>90% pure; Fig. 1) was prepared from the cell lysate with anti-flag M2 agarose beads (Sigma A2220) and DEAE-sepharose (Cytiva 17070910) (Fig. 1)
Structure	tag-hLig1 is a 103kDa protein carries 919aa. of the full length human Lig1 and the N-terminal 12 peptide including DYKDDDDK. This protein has several post-translational modifications as endogenous Lig1 in human cells and appears as multiple bands around 140kDa region in SDS-PAAG (Fig. 1)
Activity	DNA ligation activity was observed in the same reaction condition as T4DNA Ligase (02-050), but tag-hLig1 has less than 1/10 specific activity of T4DNA ligase. For example, the specific activity tag-hLig1 for HindIII digested DNA was estimated as 2U (T4ligase units)/µg and for HaeIII digested DNA as 0.5U (T4ligase units)/µg (Fig. 2).
Application	1. DNA ligation; This enzyme exhibits DNA ligase activity in the same assay condition as T4 DNA ligase (buffers and substrates). It can ligate DNA fragments with both cohesive DNA ends and blunt DNA ends similarly as T4 DNA ligase (Fig. 2). 2. Molecular interaction with PCNA. (Fig. 3) 3. Evaluation of anti-cancer reagents as inhibitors of hLig1
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 (ref.1). Several Lig1-interacting proteins have been identified. One of the interactors is DNA-sliding clamp, PCNA, involved in DNA replication and repair. The interaction with PCNA is mediated by two conserved PCNA-binding motifs, PIP box, at the N terminal and the DNA binding motif of Lig1, and involved in Okazaki fragment joining during DNA replication (ref. 2).
Data Link	UniProtKB - P18858 (DNLI1_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: 10-141 human Ligase 1 tagged with DYKDDDDK-peptide (tag-hLig1)

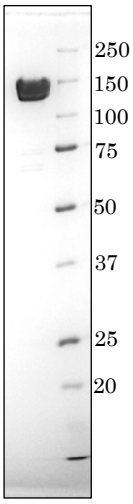

Fig 1. CBB staining of 2.4µg purified human Ligase 1

The highly purified tag-hLig1 (>90% pure) was electrophoresed in 12.5% PAAG and stained with CBB. The broad bands appeared at the higher molecular mass than its original size (103kDa) was due to multiple post-translational modifications in human cells

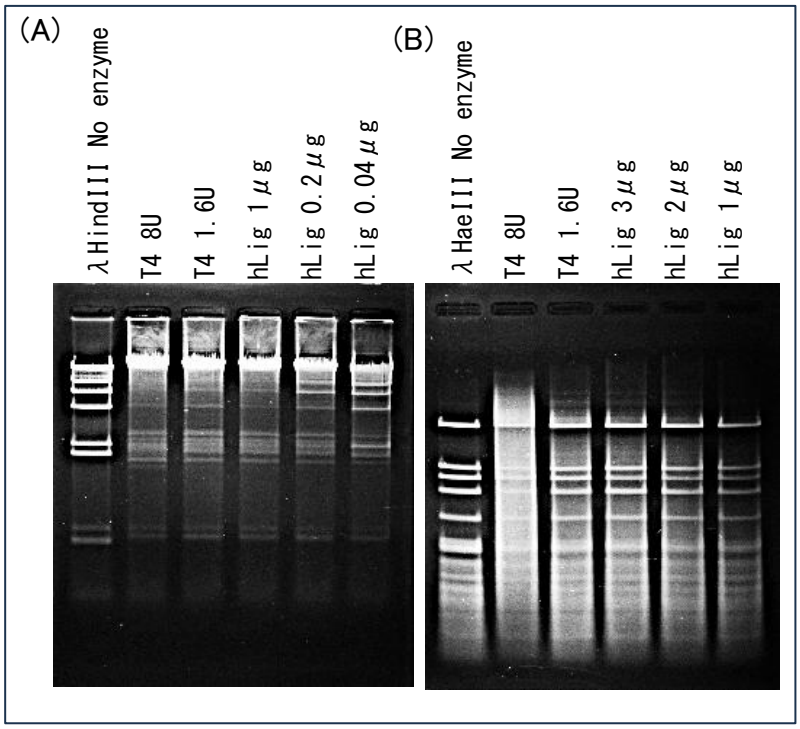

Fig 2. DNA ligation activity of human Lig1

(A) Cohesive DNA ends ligation. 2µg of HindIII-digested lambda DNA was incubated in a 10µl reaction mixture (50mM Tris-HCl pH7.6, 10mM $MgCl_2$, 1mM ATP, 10mM dithiothreitol; 02-T4b) with indicated amounts of DNA ligases at 25℃ for 30min. After the reaction, 30µl of stopping solution (1% SDS, 10% glycerol, 0.01% bromophenol blue and 100µg/ml proteinaseK) was added and incubated at 60℃ for 10min. A half of the samples (20µl) were electrophoresed in 1% agarose gel. Band-shift profiles of the substrate DNA were compared with those of T4DNA ligase with known activity units. The hLig1 activity was estimated as 2U (T4ligase units)/µg, which is less than 1/10 of T4DNA ligase.

(B) Blunt DNA ends Ligation. 1µg of HaeIII-digested lambda DNA was incubated in a 10µl reaction mixture with indicated amounts of DNA ligases at 25℃ for 180min. After the reaction, band-shift profiles were analyzed as (A) and the hLig1 activity for blunt DNA ends was estimated as 0.5U (T4ligase units)/µg.

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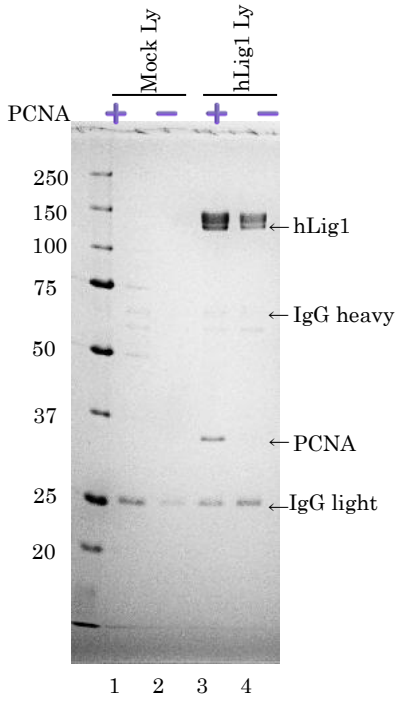

Fig. 3 Molecular interaction with PCNA

10µl of anti-flag antibody beads (M2; Sigma A2220) were pre-incubated with 293T cell mock lysate (25µl) (tubes 1 and 2) or the same cell lysate expressing tag-hLig (25µl) (tubes 3 and 4), respectively. After washing of the beads, 1.6µg PCNA was added to tubes 1 and 3 or blank buffer to tubes 2 and 4, and incubated at 4℃ for 3hrs. Unbound materials were washed off and remained proteins were eluted with 20µl of SDS loading buffer. 2.5µl each of the sample was electrophoresed in 12.5% PAAG and stained with CBB. The PCNA band specifically bound to hLig1 was observed in Lane 3. Positions of hLig1, IgG heavt chain, PCNA and light chain were indicated with arrows.

Related products:

10-151 human PCNA

70-090 Anti-DNA ligase1 (human) antibody, rabbit polyclonal

02-050 T4 DNA Ligase with reaction buffer (02-T4b)

References :

1. Eukaryotic DNA ligases: structural and functional insights. Ellenberger T, Tomkinson AE. Annu Rev Biochem. 2008; 77: 313–338, PMID: **18518823**, PMCID: PMC2933818 , doi: 10.1146/annurev.biochem.77.061306.123941
2. Mechanism of human Lig1 regulation by PCNA in Okazaki fragment sealing. Blair K, Tehseen M, Raducanu VS, Shahid T, Lancey C, Rashid F, Crehuet R, Hamdan SM, De Biasio A. Nat Commun. 2022, 13, 7833. doi: 10.1038/s41467-022-35475-z. PMID: 36539424

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