Human RPA protein, His_6-tagged (HishRPA), functional

Product code	02-046
Size	50 μg
Storage	-20℃
Concentration	1.0 mg/ml
Buffer	20mM Hepes NaOH (pH8.0), 1mM EDTA, 0.01% Igepal CA-630, 0.3M NaCl, 50% glycerol, 1mM DTT, 0.1mM PMSF, 2μg/ml leupeptine
Preparation scheme and purity	Human 293T cell was transfected with plasmid DNA mixture arranged to express three subunits of full-length human RPA, hRPA1-3. Among the subunits, RPA1 was tagged with $(His)_6$ peptide (His tag) at its N-terminal. The highly purified HishRPA (>95% pure; Fig. 1) was prepared from the cell lysate with DEAE-sepharose (Cytiva 17070910), Ni-NTA agarose (Qiagen) and ssDNA cellulose (inhouse prepared) (Fig. 1).
Activity	ssDNA specific binding was observed with the obtained HishRPA preparation by electrophoresis mobility shift assay (EMSA) with 1% agarose gel. (Fig. 2).
Application	1. ssDNA binding assay (Fig. 2). 2. Biochemical assay material for reactions of replication, repair recombination and DNA damage responses. 3. Pull down assay for RPA interacting proteins.
Background	RPA (replication protein A) is in the heterotrimeric complex, consisting of p70 (RPA1; HGNC:10289), p34 (RPA2; HGNC:10290) and p14 (RPA3; HGNC:10291) subunits, and highly conserved among eukaryotes. RPA is identified as an essential protein for DNA replication of SV40 virus *in vitro,* is also called RFA (replication factor A) or HSSP (human single-stranded DNA binding protein) (PNAS 1987, 84, 1834-8, EMBO J 1988, 7, 1211-8, PNAS 1988, 85, 2523-7). This protein binds to single-stranded DNA (ssDNA), forming a nucleoprotein complex, which protects the ssDNA from nucleases, prevents formation of secondary structures and coordinates the recruitment and departure of different genome maintenance factors. Thus, it is required for multiple processes in eukaryotic DNA metabolism including DNA replication, repair, recombination, telomere maintenance (ref. 1, 2), and coordinating the cellular response to DNA damage through activation of the ataxia telangiectasia and Rad3-related protein (ATR) kinase In cells, RPA2 is phosphorylated by DNA-dependent protein kinase when RPA is bound to single-stranded DNA (during S phase and after DNA damage; ref. 3).
Data Link	UniProt P27694 (RFA1_HUMAN), P15927 (RFA2_HUMAN), P35244 (RFA3_HUMAN)

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

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Images: 02-046 Human RPA protein, His6-tagged (HishRPA) functional

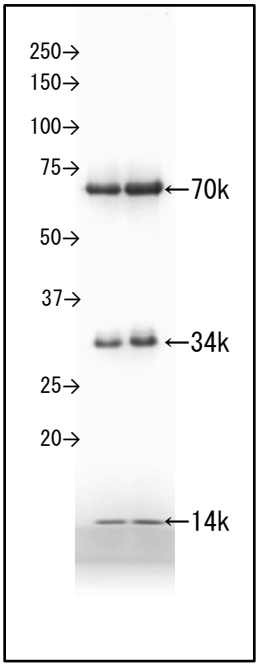

Fig. 1 Purified HisRPA

3.2µg (left) or 6.4µg (right) of purified HishRPA was separated on 12.5% SDS-PAAG and stained with CBB. Arrows at the right with molecular mass (kDal) indicate three subunits of RPA, respectively.

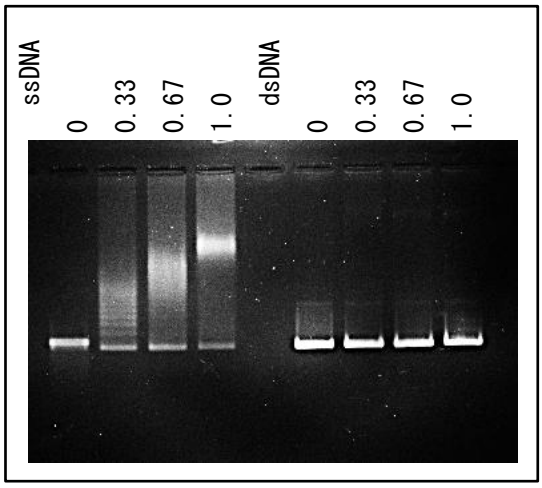

Fig. 2 Detection of ssDNA specific binding activity of HishRPA by EMSA in 1% agarose gel

A substrate 0.9kb DNA fragment was prepared by PCR amplification. 0.1µg of the fragment heated at 98℃ (ssDNA) or not (dsDNA) was incubated with indicated amounts of HishRPA in 10µl reaction mixture (10mM TrisHCl pH7.5, 10mM $MgCl_2$, 50mM NaCl) on ice for 10min. The reacted products were electrophoresed in 1% agarose gel in TAE buffer for 30min at 100V and stained with GelRed (BTI Biotium, 41003-T). According to the amounts of HishRPA, ssDNA was specifically shifted upward. Note that both ss and dsDNA of 0.9kb fragments migrated at the same position without HishRPA, but ssDNA was less stained under the used condition.

References :

1. Replication Protein A, the Main Eukaryotic Single-Stranded DNA Binding Protein, a Focal Point in Cellular DNA Metabolism Nasheuer HP, Meaney AM, Hulshoff T, Thiele I, Onwubiko NO. Int J Mol Sci. 2024 25 588. doi: 10.3390/ijms25010588 PMCID: PMC10779431
2. Replication protein A: a multifunctional protein with roles in DNA replication, repair and beyond Dueva R, Iliakis G. NAR Cancer. 2020 2 doi: 10.1093/narcan/zcaa022 PMCID: PMC8210275
3. Replication-mediated DNA damage by camptothecin induces phosphorylation of RPA by DNA-dependent protein kinase and dissociates RPA:DNA-PK complexes ShaoRG, Cao CX, Zhang H, Kohn KW, Wold MS, Pommier Y. EMBO J. 1999 18 1397–1406. doi:10.1093/emboj/18.5.1397 PMCID: PMC1171229

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