

Anti-RPA (human) antibody, rabbit polyclonal

Product code	70-085
Size	100 µg
Storage	-20°C
Concentration	1.0 mg/ml
Buffer	PBS- with 50% glycerol
Purity	IgG fraction purified with protein A from rabbit serum immunized with human RPA (Fig. 1)
Immunogen	Native RPA complex purified from human 293T cells with ssDNA agarose
Reactivity	This antibody detects human RPA2 prominently, RPA1 weakly but RPA3 hardly with human cell lysates by immunoblotting (Fig. 2). Other species have not been tested.
Validation	Specificity has been validated by immunoblotting with immunoprecipitated samples (Fig. 3)
Application	 Western blotting (1 μg/ml; Fig.2) IF (1 μg/ml; Fig.4) Immunoprecipitation (1 μg/assay with ProteinG Plus Agarose). This antibody can precipitate RPA complex from human 293T cell lysate (Fig. 3).
Background	RPA (replication protein <u>A</u>) is in the heterotrimeric complex, consisting of p70 (RPA1; <u>HGNC:10289</u>), p34 (RPA2; <u>HGNC:10290</u>) and p14 (RPA3; <u>HGNC:10291</u>) subunits, and highly conserved among eukaryotes. RPA is identified as an essential protein for DNA replication of SV40 virus <i>in vitro</i> , is also called RFA (replication factor A) or HSSP (human single-stranded DNA binding protein) (<u>PNAS 1987, 84, 1834-8</u> , <u>EMBO J 1988, 7, 1211-8</u> , <u>PNAS 1988, 85, 2523-7</u>). 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 <u>P27694</u> (RFA1_HUMAN) , <u>P15927</u> (RFA2_HUMAN) , <u>P35244</u> (RFA3_HUMAN)
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Data Images: 70-085Anti-RPA rabbit polyclonal antibody



Fig. 1 Purified anti-RPA antibody

10µg of purified anti-RPA antibody (IgG fraction) was separated on 12.5% SDS-PAAG and stained with CBB. Arrows indicate IgG heavy and light chains, respectively.



Fig. 2 Detection of endogenous RPA in whole cell extract of human 293T cells

20µg of the 293T cell lysate was separated on 12.5% SDS-PAAG and blotted on PVDF membrane with a wet-transfer apparatus at 15V overnight. The anti-RPA antibody was used at 1µg/ml for 145min, and Goat Anti-Rabbit IgG H&L (HRP) (ab205718) was applied at 1/10,000 dilution at RT for 90min. Reacted antibody was detected with Immunostar zeta reagent (291-72401, wako). The lower arrow indicates RPA2 band (34kDa) and the upper one is the RPA1 band (70kDa). The RPA3 band (14kDa) was hardly detected with this antibody.



Fig. 3 immunoprecipitation of endogenous RPA from human 293T cell lysate

500µg of the 293T cell lysate was mixed with 1µg anti-RPA antibody and incubated at 4°C for 60min. Then, incubated further with 20µl of ProteinG Plus Agarose at 4°C for 60min.

25% of the precipitated samples were applied on 12.5% SDS-PAAG and the reacted bands were detected as Fig. 2.

Lane 1: 34µg of 293T cell lysate

Lane 2: Immunoprecipitated sample from the cell lysate without antibody

Lane 3: Immunoprecipitated sample from the cell lysate with 1µg anti-RPA antibody

Lane 4: Immunoprecipitated sample only with 1µg anti-RPA antibody RPA1 and 2 bands were detected in Lane 3, precipitated with anti-RPA antibody.



Fig. 4 Immunofluorescence detection of HeLa cell nuclei

Hela cell grown in a dish was fixed with 4% PFA at 4°C and permeabilized with 0.25 % Triton X-100 in PBS- for 10 min. 1µg/ml of anti-RPA antibody was applied to the sample at 4°C overnight after blocking with BSA in PBS at RT for 75min. The reacted antibody was detected with

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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 with Nikon LED (Ts2) system (Filter cube; C-LED470). The nuclei were stained specifically with this antibody.

Related products: Not applicable

References :

- <u>Replication Protein A, the Main Eukaryotic Single-Stranded DNA Binding Protein, a Focal Point in</u> <u>Cellular DNA Metabolism</u> Nasheuer HP, Meaney AM, Hulshoff T, Thiele I, Onwubiko NO. Int J Mol Sci. 2024 25 588. doi: 10.3390/ijms25010588 PMCID: PMC10779431
- 2. <u>Replication protein A: a multifunctional protein with roles in DNA replication, repair and beyond</u> Dueva R, Iliakis G. NAR Cancer. 2020 2 doi: 10.1093/narcan/zcaa022 PMCID: PMC8210275
- <u>Replication-mediated DNA damage by camptothecin induces phosphorylation of RPA by DNA-dependent protein kinase and dissociates RPA:DNA-PK complexes</u> 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