Background
Saccharomyces cerevisiae replication protein A (RPA), also known as replication factor \(\text{A (RFA)}\) is a single-stranded DNA-binding protein that is required for multiple processes in eukaryotic DNA metabolism. Those processes include DNA replication, DNA repair, and recombination. Homologues to RPA have been identified in all eukaryotic organisms examined. RPA is heterotrimeric protein composed of subunits of approximately 70, 30, and 14 kDa. Members of this family bind nonspecifically to single-stranded DNA and interact with and/or modify the activities of multiple proteins. Alternative names: Replication protein A 69 kDa DNA-binding subunit, Single-stranded DNA-binding protein, DNA-binding protein BUF2, replication protein A 36 kDa subunit, DNA-binding protein BUF1 antibody.

Immunogen
RPA from \(\text{Saccharomyces cerevisiae}\) consisting of three subunits \(\text{RFA1 (70 kDa), RFA2 (30 kDa) and RFA3 (14 kDa)}\); overexpressed in \(\text{E.coli}\) and purified by chromatography; no affinity tags were added to any of three subunits.

Host
Rabbit

Clonality
Polyclonal

Purity
Serum

Format
Lyophilized

Quantity
50 µl

Reconstitution
For reconstitution add 50 µl of sterile water

Storage
Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.

Tested applications
Immunoprecipitation (IP), Chromatin Immunoprecipitation (ChIP), Western blot (WB)

Application information

Recommended dilution
ChIP, 1 : 20 000 (WB)

Expected | apparent MW
70 + 30 + 14 kDa

Confirmed reactivity
\(\text{Saccharomyces cerevisiae}\)

Predicted reactivity
\(\text{Saccharomyces cerevisiae}\)

Not reactive in
No confirmed exceptions from predicted reactivity are currently known.

Additional information
Antibody was also successfully used in ChIP application Holstein et al. (2014).

Load of 1 ng of the protein will allow to visualize two subunits of RPA, while load of 5 ng will allow to visualize all three subunits in Western blot technique.

Selected references
Holstein et al. (2014). Interplay between Nonsense-Mediated mRNA Decay and DNA Damage Response Pathways.


Application example

TCA precipitated protein extracts from a wild type yeast strain (S. cerevisiae) were separated on 10% gel and transferred to a PVDF membrane. Antibody was used in different dilutions: 1: 5000 (1); 1: 10 000 (2); 1: 20 000 (3);

Besides the bands for RFA1 and RFA2 an unspecific band was detected at ~150 kDa.