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Anti-RPA70 RPA1 Antibody Picoband™ (monoclonal, 11H4)

Catalog # ABO14822

Product Information

Application	WB, IHC, IF, FC
Primary Accession	P27694
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human, Mouse, Monkey
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-RPA70 RPA1 Antibody Picoband™ (monoclonal, 11H4) . Tested in Flow Cytometry, IF, IHC, WB applications. This antibody reacts with Human, Monkey, Mouse.

Additional Information

Gene ID	6117
Other Names	Replication protein A 70 kDa DNA-binding subunit, RP-A p70, Replication factor A protein 1, RF-A protein 1, Single-stranded DNA-binding protein, Replication protein A 70 kDa DNA-binding subunit, N-terminally processed, RPA1, REPA1, RPA70
Calculated MW	68138
Application Details	Western blot, 0.1-0.5 □g/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml Immunofluorescence, 2 □g/ml Flow Cytometry, 1-3 □g/1x10^6 cells
Subcellular Localization	Nucleus
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Clone Names	Clone: 11H4
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human RPA70, different from the related mouse sequence by three amino acids.
Cross Reactivity	No cross-reactivity with other proteins.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C

for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

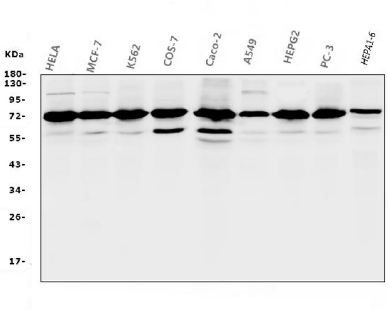
Name	RPA1
Synonyms	REPA1, RPA70
Function	As part of the heterotrimeric replication protein A complex (RPA/RP-A), binds and stabilizes single-stranded DNA intermediates that form during DNA replication or upon DNA stress. It prevents their reannealing and in parallel, recruits and activates different proteins and complexes involved in DNA metabolism (PubMed:17596542, PubMed:27723717, PubMed:27723720). Thereby, it plays an essential role both in DNA replication and the cellular response to DNA damage (PubMed:9430682). In the cellular response to DNA damage, the RPA complex controls DNA repair and DNA damage checkpoint activation. Through recruitment of ATRIP activates the ATR kinase a master regulator of the DNA damage response (PubMed:24332808). It is required for the recruitment of the DNA double-strand break repair factors RAD51 and RAD52 to chromatin in response to DNA damage (PubMed:17765923). Also recruits to sites of DNA damage proteins like XPA and XPG that are involved in nucleotide excision repair and is required for this mechanism of DNA repair (PubMed:7697716). Also plays a role in base excision repair (BER) probably through interaction with UNG (PubMed:9765279). Also recruits SMARCAL1/HARP, which is involved in replication fork restart, to sites of DNA damage. Plays a role in telomere maintenance (PubMed:17959650, PubMed:34767620). As part of the alternative replication protein A complex, aRPA, binds single-stranded DNA and probably plays a role in DNA repair. Compared to the RPA2- containing, canonical RPA complex, may not support chromosomal DNA replication and cell cycle progression through S-phase. The aRPA may not promote efficient priming by DNA polymerase alpha but could support DNA synthesis by polymerase delta in presence of PCNA and replication factor C (RFC), the dual incision/excision reaction of nucleotide excision repair and RAD51-dependent strand exchange (PubMed:19996105). RPA stimulates 5'-3' helicase activity of the BRIP1/FANCJ (PubMed:17596542).
Cellular Location	Nucleus. Nucleus, PML body. Note=Enriched in PML bodies in cells displaying alternative lengthening of their telomeres

Background

Replication protein A 70 kDa DNA-binding subunit is a protein that in humans is encoded by the RPA1 gene. This gene is mapped to chromosome 17p13.3. Replication protein A (RPA) is a heterotrimeric single-strand DNA (ssDNA)-binding protein essential for DNA replication, repair, and recombination. It is composed of 70-kD (RPA1), 32-kD (RPA2), and 14-kD (RPA3) subunits. The RPA1 subunit is responsible for high-affinity ssDNA binding. The RPA complex was originally isolated as a factor essential for in vitro replication of the papovavirus SV40. It had been found that recombinant human RPA1, purified from bacteria, exhibited ssDNA-binding activity comparable to that of the complete RPA complex. RPA1 could substitute for the complete complex in stimulating the activity of DNA polymerase alpha-primase, but it could not substitute for the complete complex in SV40 DNA replication in vitro, suggesting an important functional role for the other subunits.

Images

Figure 1. Western blot analysis of RPA70 using anti-RPA70



antibody (M01317-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: HELA whole cell lysates,
Lane 2: MCF-7 whole cell lysates,
Lane 3: K562 whole cell lysates,
Lane 4: COS-7 whole cell lysates,
Lane 5: Caco-2 whole cell lysates,
Lane 6: A549 whole cell lysates,
Lane 7: HEPG2 whole cell lysates,
Lane 8: PC-3 whole cell lysates,
Lane 9: HEPA1-6 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-RPA70 antigen affinity purified monoclonal antibody (Catalog # M01317-2) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for RPA70 at approximately 12KD. The expected band size for RPA70 is at 12KD.

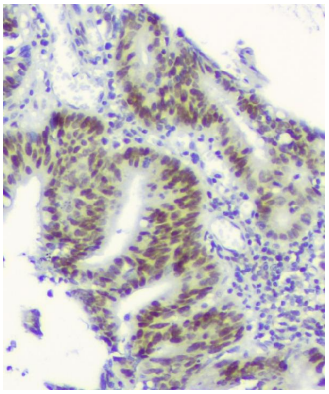


Figure 2. IHC analysis of RPA70 using anti-RPA70 antibody (M01317-2).
RPA70 was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-RPA70 Antibody (M01317-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

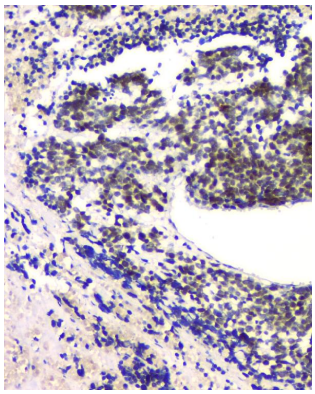
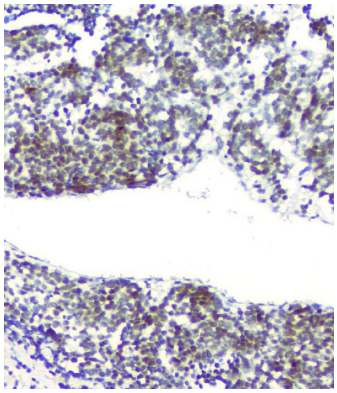


Figure 3. IHC analysis of RPA70 using anti-RPA70 antibody (M01317-2).
RPA70 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-RPA70 Antibody (M01317-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

Figure 4. IHC analysis of RPA70 using anti-RPA70 antibody (M01317-2).



RPA70 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-RPA70 Antibody (M01317-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

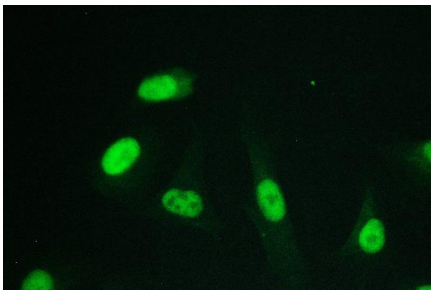


Figure 5. IF analysis of RPA70 using anti-RPA70 antibody (M01317-2).
RPA70 was detected in immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-RPA70 Antibody (M01317-2) overnight at 4°C. DyLight488 Conjugated Goat Anti-mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

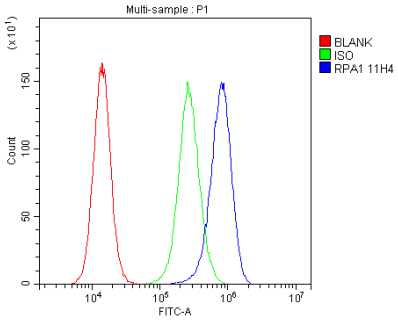


Figure 6. Flow Cytometry analysis of A431 cells using anti-RPA70 antibody (M01317-2).
Overlay histogram showing A431 cells stained with M01317-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-RPA70 Antibody (M01317-2,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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