10320 Camino Santa Fe, Suite G
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Anti-RPA32/RPA2 Antibody Picoband™ (monoclonal, 3B2E9)

Catalog # ABO15081

Specification

Anti-RPA32/RPA2 Antibody Picoband™ (monoclonal, 3B2E9) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	P15927
Host	**Mouse**
Isotype	**Mouse IgG2a**
Reactivity	**Human**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-RPA32/RPA2 Antibody Picoband™ (monoclonal, 3B2E9) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.

Reconstitution
Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Anti-RPA32/RPA2 Antibody Picoband™ (monoclonal, 3B2E9) - Additional Information

Gene ID 6118

Other Names
Replication protein A 32 kDa subunit, RP-A p32, Replication factor A protein 2, RF-A protein 2, Replication protein A 34 kDa subunit, RP-A p34, RPA2, REPA2, RPA32, RPA34

Calculated MW
32 kDa KDa

Application Details
Western blot, 0.25-0.5 µg/ml, Human
 Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human
 Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Contents
Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.

Immunogen
E.coli-derived human RPA32/RPA2 recombinant protein (Position: Q34-H254).

Purification
Immunogen affinity purified.

Storage	**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 freezing and thawing.**

Anti-RPA32/RPA2 Antibody Picoband™ (monoclonal, 3B2E9) - Protein Information

Name RPA2

Synonyms REPA2, RPA32, RPA34

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. Thereby, it plays an essential role both in DNA replication and the cellular response to DNA damage. 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. It is required for the recruitment of the DNA double-strand break repair factors RAD51 and RAD52 to chromatin in response to DNA damage. 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. Also plays a role in base excision repair (BER) probably through interaction with UNG. Also recruits SMARCAL1/HARP, which is involved in replication fork restart, to sites of DNA damage. May also play a role in telomere maintenance. RPA stimulates 5'-3' helicase activity of BRIP1/FANCJ (PubMed:17596542).

Cellular Location
Nucleus. Nucleus, PML body. Note=Redistributes to discrete nuclear foci upon DNA damage in an ATR-dependent manner

Anti-RPA32/RPA2 Antibody Picoband™ (monoclonal, 3B2E9) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-RPA32/RPA2 Antibody Picoband™ (monoclonal, 3B2E9) - Images

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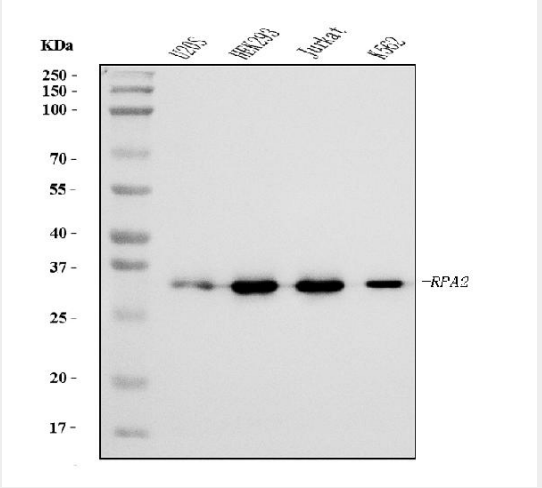

Figure 1. Western blot analysis of RPA32/RPA2 using anti-RPA32/RPA2 antibody (M02067-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human U20S whole cell lysates,
Lane 2: human HEK293 whole cell lysates,
Lane 3: human Jurkat whole cell lysates,
Lane 4: human K562 whole cell lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA 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-RPA32/RPA2 antigen affinity purified monoclonal antibody (Catalog # M02067-2) 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 1:10000 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 RPA32/RPA2 at approximately 32 kDa. The expected band size for RPA32/RPA2 is at 32 kDa.

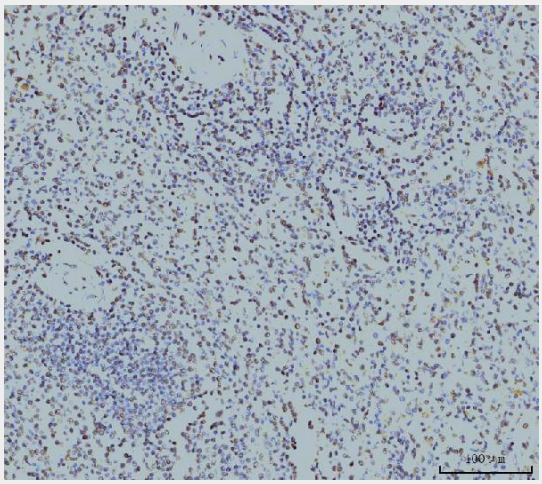

Figure 2. IHC analysis of RPA32/RPA2 using anti-RPA32/RPA2 antibody (M02067-2).
RPA32/RPA2 was detected in a paraffin-embedded section of human spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-RPA32/RPA2 Antibody (M02067-2) 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.

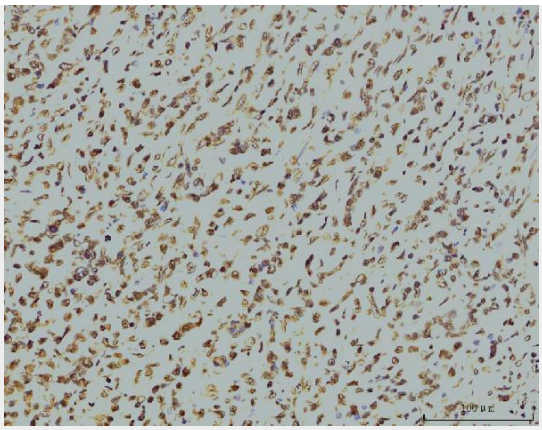

Figure 3. IHC analysis of RPA32/RPA2 using anti-RPA32/RPA2 antibody (M02067-2).
RPA32/RPA2 was detected in a paraffin-embedded section of human gastric cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-RPA32/RPA2 Antibody (M02067-2) 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.

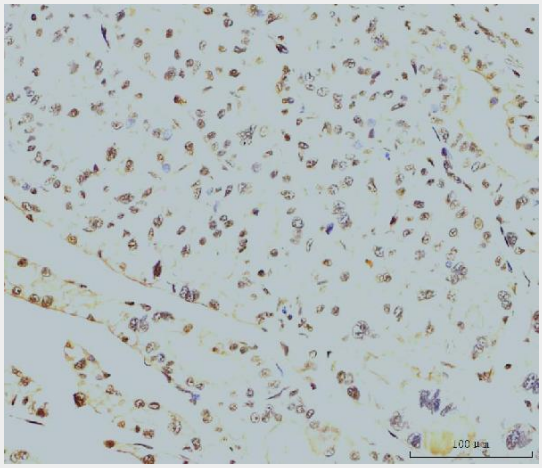

Figure 4. IHC analysis of RPA32/RPA2 using anti-RPA32/RPA2 antibody (M02067-2).
RPA32/RPA2 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-RPA32/RPA2 Antibody (M02067-2) 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.

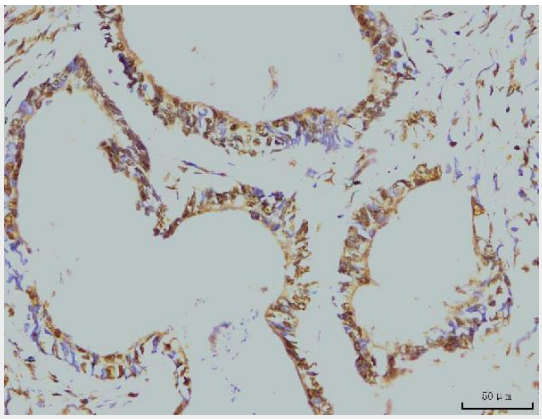

Figure 5. IHC analysis of RPA32/RPA2 using anti-RPA32/RPA2 antibody (M02067-2).
RPA32/RPA2 was detected in a paraffin-embedded section of human colonic adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-RPA32/RPA2 Antibody (M02067-2) 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.

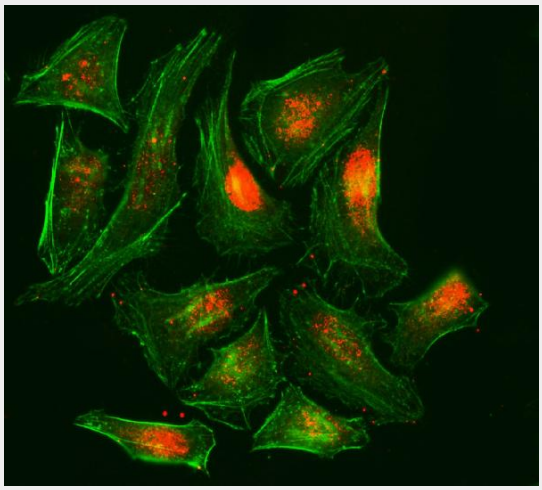

Figure 6. IF analysis of RPA32/RPA2 using anti-RPA32/RPA2 antibody (M02067-2).
RPA32/RPA2 was detected in an immunocytochemical section of Hela cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-RPA32/RPA2 Antibody (M02067-2) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Mouse IgG (BA1141) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The tissue section was developed using Phalloidin-iFluor 488 Conjugated. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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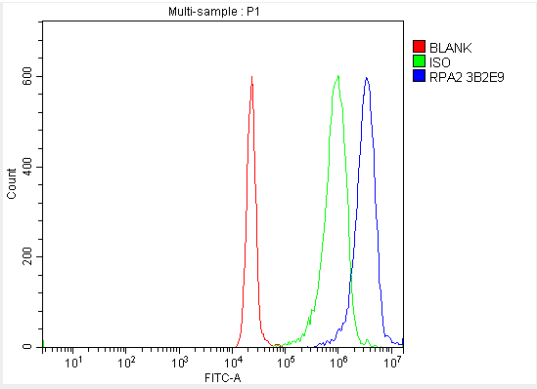

Figure 7. Flow Cytometry analysis of JK cells using anti-RPA32/RPA2 antibody (M02067-2). Overlay histogram showing JK cells stained with M02067-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-RPA32/RPA2 Antibody (M02067-2, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 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.

Anti-RPA32/RPA2 Antibody Picoband™ (monoclonal, 3B2E9) - Background

This gene encodes a subunit of the heterotrimeric Replication Protein A (RPA) complex, which binds to single-stranded DNA (ssDNA), forming a nucleoprotein complex that plays an important role in DNA metabolism, being involved in DNA replication, repair, recombination, telomere maintenance, and co-ordinating the cellular response to DNA damage through activation of the ataxia telangiectasia and Rad3-related protein (ATR) kinase. The RPA complex protects single-stranded DNA from nucleases, prevents formation of secondary structures that would interfere with repair, and co-ordinates the recruitment and departure of different genome maintenance factors. The heterotrimeric complex has two different modes of ssDNA binding, a low-affinity and high-affinity mode, determined by which oligonucleotide/oligosaccharide-binding (OB) domains of the complex are utilized, and differing in the length of DNA bound. This subunit contains a single OB domain that participates in high-affinity DNA binding and also contains a winged helix domain at its carboxy terminus, which interacts with many genome maintenance protein. Post-translational modifications of the RPA complex also plays a role in co-ordinating different damage response pathways.