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Anti-Replication factor C subunit 1 RFC1 Antibody

Catalog Number: PA1716

About RFC1

RFC (Replication Factor C), also called activator-1, is a multimeric primer-recognition protein consisting of 5 distinct subunits of 145, 40, 38, 37, and 36.5 kD. Human RFC was purified from extracts of HeLa cells as a host factor essential for the in vitro replication of simian virus 40 (SV40) DNA. RFC, in the presence of ATP, assembles proliferating-cell nuclear antigen and DNA polymerase-delta or polymerase-epsilon on primed DNA templates. Tight interfacial coordination of the ATP analog ATP-gamma-S by RFC resulted in a spiral arrangement of the ATPase domains of the clamp loader above the PCNA ring.

Overview

Product Name	Anti-Replication factor C subunit 1 RFC1 Antibody
Reactive Species	Human
Description	Boster Bio Anti-Replication factor C subunit 1 RFC1 Antibody catalog # PA1716. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg Thimerosal, 0.01mg NaN3.
Storage Instructions	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.
Host	Rabbit
Uniprot ID	P35251

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human RFC1.
Predicted Reactive Species	Hamster
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.

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Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.1-0.5ug/ml, Human Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, By Heat Immunocytochemistry/Immunofluorescence, 5 ug/ml, Human Flow Cytometry(Fixed), 1-3 ug/1x10^6 cells, Human

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Anti-Replication factor C subunit 1 RFC1 Antibody (PA1716) Images

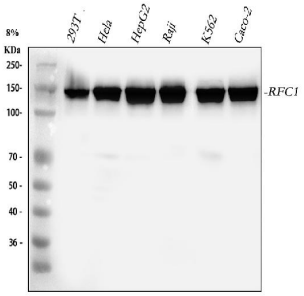


Figure 1. Western blot analysis of RFC1 using anti-RFC1 antibody (PA1716).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human 293T whole cell lysates,
Lane 2: human Hela whole cell lysates,
Lane 3: human HepG2 whole cell lysates,
Lane 4: human Raji whole cell lysates,
Lane 5: human K562 whole cell lysates,
Lane 6: human CACO-2 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 rabbit anti-RFC1 antigen affinity purified polyclonal antibody (Catalog # PA1716) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit 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 # EK1002) with Tanon 5200 system. A specific band was detected for RFC1 at approximately 150 kDa. The expected band size for RFC1 is at 128 kDa.

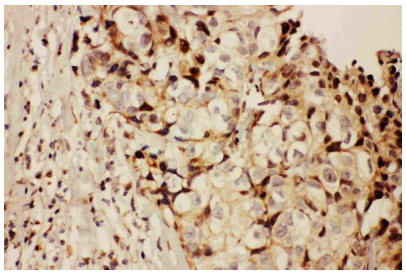


Figure 2. IHC analysis of RFC1 using anti-RFC1 antibody (PA1716).
RFC1 was detected in paraffin-embedded section of human mammary cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-RFC1 Antibody (PA1716) overnight at 4°C. Biotinylated goat anti-rabbit 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 # SA1022) with DAB as the chromogen.

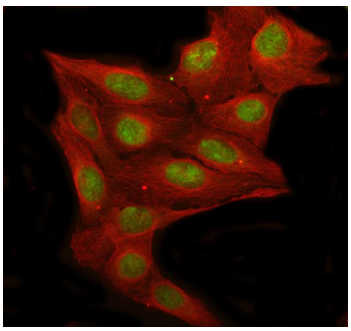


Figure 3. IF analysis of RFC1 using anti-RFC1 antibody (PA1716) and anti-Beta Tubulin antibody (M01857-3).
RFC1 was detected in immunocytochemical section of U2OS cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 ug/mL rabbit anti-RFC1 Antibody (PA1716) and mouse anti-Beta Tubulin antibody (M01857-3) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) and DyLight®594 Conjugated Goat Anti-Mouse IgG (BA1141) were used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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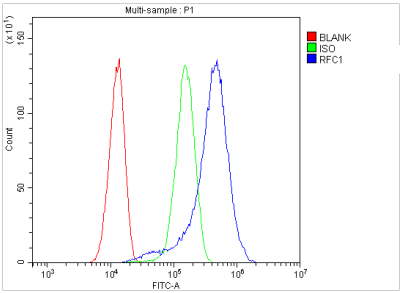


Figure 4. Flow Cytometry analysis of Hela cells using anti-RFC1 antibody (PA1716).
Overlay histogram showing Hela cells stained with PA1716 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-RFC1 Antibody (PA1716, 1 ug/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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