Revision 5

Store at -20C

#54762

Phospho-RPA32/RPA2 (Ser8) (E5A2F) Rabbit mAb

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IF-IC, FC-FP	H	Endogenous	32	Rabbit IgG	#P15927	6118

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunofluorescence (Immunocytochemistry)	1:200 - 1:800
Flow Cytometry (Fixed/Permeabilized)	1:400 - 1:1600

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity/Sensitivity

Phospho-RPA32/RPA2 (Ser8) (E5A2F) Rabbit mAb recognizes endogenous levels of RPA32/RPA2 protein only when phosphorylated at Ser8.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser8 of human RPA32/RPA2 protein.

Background

RPA70 (HSSB, REPA1, RF-A, RP-A, p70) is a component of a heterotrimeric complex, composed of 70, 32/30, and 14 kDa subunits, collectively known as RPA. RPA is a single-stranded DNA binding protein, whose DNA binding activity is believed to reside entirely in the 70 kDa subunit. The complex is required for almost all aspects of cellular DNA metabolism such as DNA replication (1-3), recombination, cell cycle and DNA damage checkpoints, and all major types of DNA repair including nucleotide excision, base excision, mismatch, and double-strand break repairs (4-7). In response to genotoxic stress in eukaryotic cells, RPA has been shown to associate with the Rad9/Rad1/Hus1 (9-1-1) checkpoint complex (8). RPA is hyperphosphorylated upon DNA damage or replication stress by checkpoint kinases including ataxia telangiectasia mutated (ATM), ATM and Rad3-related (ATR), and DNA-dependent protein kinase (DNA-PK) (9-11). Phosphorylation of RPA32 occurs at serines 4, 8, and 33 (11). Hyperphosphorylation may alter RPA-DNA and RPA-protein interactions. In addition to the checkpoint partners, RPA interacts with a wide variety of protein partners, including proteins required for normal replication such as RCF, PCNA, and Pol α, and also proteins involved in SV40 replication, such as DNA polymerase I and SV40 large T antigen (10,12).

Background References

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11. Nuss, J.E. et al. (2005) *Biochemistry* 44, 8428-37.
12. Yuzhakov, A. et al. (1999) *EMBO J.* 18, 6189-99.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

H: Human

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