

RPA32 (4E4) Rat mAb

✓ 100 µl
(10 western blots)

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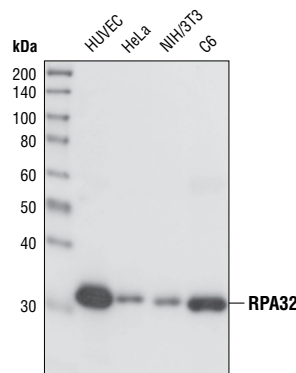
rev. 03/29/10

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC Endogenous	H, M, R, Mk, Hm	32 kDa	Rat IgG1**

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 (1,2,3), 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 (4,5,6,7).

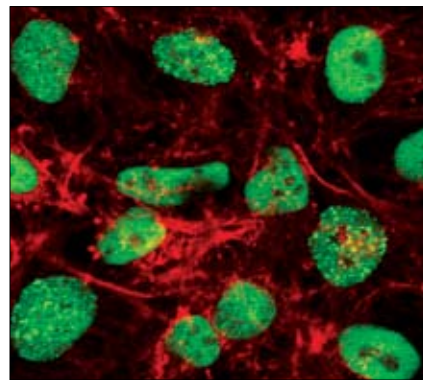
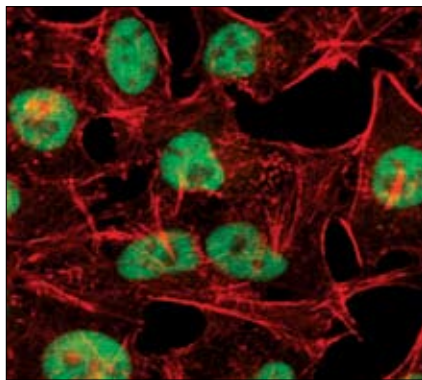
In response to genotoxic stress in eukaryotic cells, RPA has been shown to associate with the Rad9/Rad1/Hus1 (9-1-1) checkpoint complex (8). RPA is hyperphosphorylated upon DNA damage or replication stress by checkpoint kinases including ataxia telangiectasia mutated (ATM), ATM and Rad3-related (ATR), and DNA-dependent protein kinase (DNA-PK) (9,10,11). 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 (12,10).



Western blot analysis of extracts from various cell lines, using RPA32 (4E4) Rat mAb.

Specificity/Sensitivity: RPA32 (4E4) Rat mAb detects endogenous levels of total RPA32 protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with recombinant full-length human MBP-RPA32 protein.



Confocal immunofluorescent images of HeLa cells, untreated (left) or UV-treated (right), labeled with RPA32 (4E4) Rat mAb (green) showing translocation to distinct nuclear foci after UV damage. Actin filaments have been labeled with Alexa Fluor® 555 phalloidin. Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

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

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.

*Species cross-reactivity is determined by western blot.

**Anti-rat secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:100
Immunofluorescence (IF-IC)	1:200

Background References:

- (1) Liu, V.F. and Weaver, D.T. (1993) *Mol. Cell Biol.* 13, 7222-7231.
- (2) Wobbe, C.R. et al. (1987) *Proc. Natl. Acad. Sci. USA* 84, 1834-1838.
- (3) Fairman, M.P. and Stillman, B. (1988) *EMBO J.* 7, 1211-1218.
- (4) Wold, M.S. and Kelly, T. (1988) *Proc. Natl. Acad. Sci. USA* 85, 2523-2527.
- (5) Zhou, B.B. and Elledge, S.J. (2000) *Nature* 408, 433-439.
- (6) Kastan, M.B. and Bartek, J. (2004) *Nature* 432, 316-323.
- (7) Sancar, A. et al. (2004) *Annu. Rev. Biochem.* 73, 39-85.
- (8) Guo, S. et al. (2006) *J. Biol. Chem.* Epub ahead of print.
- (9) Wu, X. et al. (2005) *Oncogene* 24, 4728-4735.
- (10) Binz, S.K. et al. *DNA Repair (Amst)* 3, 1015-1024.
- (11) Nuss, J.E. et al. (2005) *Biochemistry* 44, 8428-8437.
- (12) Yuzhakov, A. et al. (1999) *EMBO J.* 18, 6189-6199.

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Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.