Revision 1

Store at -20C

#8341

DNA Replication Antibody Sampler Kit

Cell Signaling TECHNOLOGY®

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

Product Includes	Product #	Quantity	Mol. Wt	Isotype/Source
CDT1 (D10F11) Rabbit mAb	8064	40 µl	65 kDa	Rabbit IgG
MCM2 (D7G11) XP® Rabbit mAb	3619	40 µl	125 kDa	Rabbit IgG
MCM3 (D47B6) Rabbit mAb	4003	40 µl	100 kDa	Rabbit IgG
MCM7 (D10A11) XP® Rabbit mAb	3735	40 µl	80 kDa	Rabbit IgG
PCNA (PC10) Mouse mAb	2586	40 µl	36 kDa	Mouse IgG2a
RPA70/RPA1 (C24F2) Rabbit mAb	2193	40 µl	70 kDa	Rabbit IgG
p58 Primase (8D3) Rat mAb	4726	40 µl	58 kDa	Rat IgG2a
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse
Anti-rat IgG, HRP-linked Antibody	7077	100 µl		Goat

Please visit cellsignal.com for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

Description

The DNA Replication Antibody Sampler Kit provides a fast and economical means of evaluating multiple targets regulating DNA replication. The kit contains enough primary antibodies to perform four western blots with each antibody.

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.

Background

The initiation of DNA replication in mammalian cells is a highly coordinated process that is regulated by several protein complexes. Origins of replication (ORCs), at which replication is initiated, are dispersed throughout the genome. Their activities are regulated via the sequential binding of pre-replication and replication factors that initiate formation of replication forks, the active structures at which DNA is synthesized. The origin recognition complex is thought to be bound to chromatin throughout the cell cycle (1,2). The pre-replication complex (Pre-RC) forms in late mitosis/early G1 phase beginning with the binding of CDT1 and CDC6 to the origin. Together CDT1 and CDC6 promote the loading of the heterohexameric minichromosome maintenance (MCM) complex. This process is referred to as chromatin licensing. Licensing of the chromatin permits the DNA to replicate only once per cell cycle, helping to ensure that genetic alterations and malignant cell growth do not occur (reviewed in 3). The canonical MCM complex proteins (MCM2-7) are a family of six related phospho-proteins that function, in part, as the eukaryotic replicative DNA helicase (3,4). Phosphorylation and ubiquitination of the MCM2, MCM3, MCM4, and MCM6 subunits appears to regulate MCM complex activity and the initiation of DNA synthesis (5-7). MCM proteins are removed during DNA replication, causing chromatin to become unlicensed, inhibiting Pre-RC reformation. In addition to DNA polymerase, initiation of DNA replication requires a pair of primase subunits. DNA Primase activity catalyzes de novo synthesis of an RNA/DNA primer (initiator DNA) on the leading and lagging strands, while polymerase activity extends the initiator DNA (8). The 48 and 58 kDa primase subunits cooperate in the synthesis of small RNA primers. p48 is the catalytically active subunit (9), while p58 couples p48 to the polymerase to allow the transfer of primers to the active site. The p58 subunit may also play a role in regulation of primer length (10,11). Once replication is initiated, Proliferating Cell Nuclear Antigen (PCNA) serves as an accessory factor for DNA polymerases delta and epsilon, acting to tether these polymerases to template DNA during replication. Interactions of PCNA with DNA polymerases increase the processivity of leading strand synthesis. PCNA, a member of DNA sliding clamp family, is a homotrimeric ring complex that encircles and slides along the DNA double helix as the replication fork progresses (12). Multiple proteins involved in DNA replication, DNA repair, and cell cycle control bind to PCNA and regulate DNA synthesis. PCNA is loaded onto the DNA in an ATP-dependent manner by a multiprotein clamp loader, Replication Factor C (RFC) (13). RFC, in turn, associates with DNA via interactions with the single-stranded DNA binding protein complex, Replication Protein A (RPA). The canonical RPA complex is heterotrimeric and composed of RPA1 (RPA70), RPA2 (RPA32), and RPA3 (RPA14) subunits. RPA recognizes and stabilizes single stranded DNA in repair processes and DNA recombination, and plays a role in replication (14-17).

Background References

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