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DNA Primase(p49)Ab -1

Rabbit Polyclonal Antibody

Cat.#04 -10-4991,04 -10-4992,or04 -10-4990(0.1ml,0.5ml,or1.0ml at 1.0mg/ml) (Purified Ab with BSA and Azide)

Cat.#04 -10-4993or04 -10-4994(0.5ml or 1.0ml at 1.0mg/ml) (Purified Ab without BSA and Azide)

Specificity and Comments:

Recognizes a 49kDa protein, identified as the p49 subunit of DNA primase. In the eukaryotic cell, DNA primase initiates DNA replication by the synthesis of small ribonucleotides called primers. The eukaryotic primase is composed of two subunits, p49 and p58, which purify as a complex tightly bound to DNA polymerase α . The tight association of primase with DNA polymerase α implicates the DNA polymerase α as the lagging -strand DNA polymerase in replication. One unique property of primases (as well as RNA polymerases) is the ability to synthesize nucleotides *de novo* on a template by the formation of an initial dinucleotide. Primase initiates synthesis with a triphosphate purine moiety at the 5' -end. Relatively few errors are made during the formation of the dinucleotide, whereas the primase readily misincorporates ribonucleotides during elongation of this dinucleotide. After synthesis of 7 -10 ribonucleotides, the primer-template is translocated intramolecularly to the active site of the DNA polymerase α subunit. The p49 subunit of DNA primase contains the catalytic active site. Analyses of mutant proteins indicate that residues 104 -111 are most critical for primer synthesis and form part of the active part. Alanine substitution in residues Glu¹⁰⁵, Asp¹⁰⁹, and Asp¹¹¹ produces protein with no detectable activity in direct primase assays, indicating that these residues may form part of a conserved carboxylic triad also observed in the active sites of DNA polymerases and reverse transcriptases. **BIOCARTA'S** Ab -1 is raised against the p49 subunit of DNA primase and it shows no cross reaction either with the p58 subunit of DNA primase or the DNA polymerase gamma.² The human primase subunits are 90% identical in amino acid sequence to the mouse homologs.

Species Reactivity: Human and Mouse. Others not tested.

Immunogen: Purified MOUSE recombinant p49 subunit of DNA primase.¹

Supplied As:

Total IgG purified from rabbit anti -serum by Protein A chromatography. Prepared at 1mg/ml in 10mM PBS, pH 7.4, with 0.2% BSA & 15mM sodium azide.
Also available without BSA and azide at 1mg/ml.

Storage and Stability:

Store vial at 4°C. When stored at 2 -8°C, this antibody is stable for 24 months.

Applications and Suggested Dilutions:

- Immunofluorescence (Not assessed)
- Immunohistology (Not assessed)
- Immunoprecipitation (Use Protein A)
(Ab at 10µg/mg protein lysate)
- Western Blotting¹ (Ab 5µg/ml for 2hrs at RT)

The optimal dilution for a specific application should be determined by the investigator.

Positive Control: HeLa cells.

Cellular Localization: Nuclear

Limitations:

It is available for research use only and is not approved for use in humans or in clinical diagnosis.

Custom Service:

Contact us if you require this Ab in a special format.

BIOCARTA'S Other Related Antibodies:

DNA Polymerase beta, DNA Polymerase gamma, DNA Polymerase epsilon, Replication Protein A, ORC1, Cyclin A, Cyclin B1, Cyclin C, Cyclin D1, Cyclin D2, Cyclin D3, p15, p16, p18, p19, p21^{WAF1}, p27^{KIP1}, cdk1 / p34^{cdc2}, cdk2, cdk3, cdk4, cdk5, cdk6, cdk7, cdk8, PCNA, BrdU, E2F1, E2F2, E2F3, E2F4, DP1, DP2.

Key References:

1. Copeland WC. Protein Expression and Purification, 1997;9:1-9.
2. Ropp PA *et al.*, Genomics, 1996;36:449-458.

Mini Review on DNA Primase:

Primase is the ssDNA -dependent RNA polymerase that synthesizes RNA primers during DNA replication. In common with all DNA and RNA polymerases, primase has structural

and functional features involved in polymer elongation. As a primase, it has structural and functional features for initiating chain synthesis on ssDNA. Employing amino acid sequence analysis the structure of *Escherichia coli* primase responsible for binding zinc, at least three magnesium, and DnaB helicase has been identified. One of the magnesium binding motifs resembles the active magnesium motif found in all DNA and RNA polymerases. This motif is involved in phosphodiester bond formation. The region with the putative zinc binding motif is the most highly conserved portion, including more than 25% of identical residues among bacterial primases. The function of the zinc finger may be to bind ssDNA in a sequence-specific manner. Primase has RNAP motif, a sequence found in all RNA polymerases which may be involved in chain initiation. Many of the observations concerning primer synthesis initiation in vivo have been reproduced by several of the in vitro assay systems. Important among these is that Okazaki fragments are initiated in vivo from d(CTG) most of the time. This trinucleotide initiation specificity has been shown to be an intrinsic property of pure primase in vitro. Using artificial ssDNA templates, primase has been shown to be the slowest and most error-prone polymerase yet studied. The rate-determining step is the first phosphodiester bond formed. Any protein which can influence either the dinucleotide synthesis rate or primase-ssDNA binding affinity will also play a key role in the regulation of primer synthesis initiation.

The isolation and purification of a multiprotein complex for DNA replication from MDA MB -468 human breast cancer (BC) cells was carried out. This complex, which is designated as the DNA synthesome, fully supports the in vitro replication of simian virus 40 (SV40) origin-containing DNA in the presence of the viral large T-antigen. Since the SV40 virus utilizes the host's cellular proteins for its own DNA replication, results indicate that the DNA synthesome may play a role not only in viral DNA synthesis but in human breast cell DNA replication as well. Studies demonstrate that the following DNA replication proteins constitute the DNA synthesome: DNA polymerase alpha, DNA primase, DNA polymerase delta, proliferating cell nuclear antigen, replication protein A, replication factor C, DNA topoisomerases I, II, and DNA polymerase epsilon. In addition, the DNA synthesome from human BC tissue as well as from xenografts from nude mice injected with the human BC cell line MCF -7, has been successfully isolated. The DNA synthesome purified from the BC tissues fully supports SV40 DNA replication in vitro. Furthermore, results obtained from a novel forward mutagenesis assay suggest that the DNA synthesome isolated from a nonmalignant breast cell line mediates SV40 DNA replication by an error-resistant mechanism. In contrast, the DNA synthesome derived from BC cells and tissue exhibited a lower fidelity for DNA synthesis in vitro. Overall, the

experimental data support the role of the DNA synthesome as mediating breast cell DNA replication in vitro and in vivo.

Human cell extracts efficiently support replication of simian virus 40 (SV40) DNA in vitro, while mouse cell extracts do not. Since human DNA polymerase alpha-primase is the major species-specific factor, the required subunit(s) of DNA polymerase alpha-primase were defined for this species specificity. Recombinant human, mouse, and hybrid human-mouse DNA polymerase alpha-primase complexes were expressed with baculovirus vectors and purified. All of the recombinant DNA polymerase alpha-primases showed enzymatic activity and efficiently synthesized the complementary strand on an M13 single-stranded DNA template. The human DNA polymerase alpha-primase (four subunits [HHHH]) and the hybrid DNA polymerase alpha-primase HHMM (two human subunits and two mouse subunits), containing human p180 and p68 and mouse primase, initiated SV40 DNA replication in a purified system. The human and the HHMM complex efficiently replicated SV40 DNA in mouse extracts from which DNA polymerase alpha-primase was deleted, while MMMM and the MMHH complex did not. To determine whether the human p180 or p68 subunit was required for SV40 DNA replication, hybrid complexes containing only one human subunit, p180 or p68, together with three mouse subunits (HMMM and MHMM) or three human subunits and one mouse subunit (MHHH and HMHH) were tested for SV40 DNA replication activity. The hybrid complexes HMMM and HMHH synthesized oligoribonucleotides in the SV40 initiation assay with purified proteins and replicated SV40 DNA in depleted mouse extracts. In contrast, the hybrid complexes containing mouse p180 were inactive in both assays. In conclusion, the human p180 subunit determines host-specific replication of SV40 DNA in vitro.

References:

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7. Weisshart K, Taneja P, Fanning E: The replication protein Abinding site in simian virus 40 (SV40) T antigen and its role in the initial steps of SV40 DNA replication. *J Virol* 1998;72:9771-9781.
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Warranty:

There are no warranties, expressed or implied, which extend beyond this description. Biocarta is not liable for property damage, personal injury, or economic loss caused by this product.

Material Safety Data:

This product is not licensed or approved for administration to humans or to animals other than the experimental animals. Standard Laboratory Practices should be followed when handling this material. The chemical, physical, and toxicological properties of this material have not been thoroughly investigated. Appropriate measures should be taken to avoid skin and eye contact, inhalation, and ingestion. The material contains 0.1% sodium azide as a preservative. Although the quantity of azide is very small, appropriate care should be taken when handling this material as indicated above. The National Institute of Occupational Safety and Health has issued a bulletin citing the potential explosion hazard due to the reaction of sodium azide with copper, lead, brass, or solder in the plumbing systems. Sodium azide forms hydrazoic acid in acidic conditions and should be discarded in a large volume of running water to avoid deposits forming in metal drainage pipes.