Certificate of Analysis

DNA-Dependent Protein Kinase:

Part# 9PIV581

Revised 11/11

Part No.	Size
V581A	2,500 units

Description: DNA-Dependent Protein Kinase (DNA-PK) consists of an approximate 460kDa catalytic subunit and a heterodimeric DNA-binding subunit (Ku) containing a 85kDa and a 70kDa peptide (1). It is purified from HeLa cells.

Storage Buffer: 25mM HEPES (pH 7.5), 50mM KCl, 0.2mM EDTA, 10mM $MgCl_2$, 1mM DTT, 10% glycerol.

Note: The storage buffer formulation has been changed to remove the IGEPAL CA-630 detergent.

Storage Conditions: See the storage recommendations on the Product Information Label. DNA-PK is stable at 4°C for 1 hour. Avoid multiple freeze-thaw cycles or exposure to frequent temperature changes. These fluctuations can greatly alter product stability. See the expiration date on the Product Information Label.

Unit Definition: One unit is the amount of enzyme required to incorporate 1pmol of phosphate into DNA-PK Peptide Substrate (Cat.# V5671) in one minute at 30°C.

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Quality Control Assays

Activation: The enzyme activity is increased by at least tenfold in the presence of 10µg/ml of linear, double-stranded DNA.

Activity Assay Conditions: 50mM HEPES (pH 7.5), 1mM DTT, 0.1mM EDTA, 0.2mM EGTA, 10mM $MgCl_2$, 0.1M KCl, 1.14mM DNA-PK Peptide Substrate (Cat.# V5671), 80µg/ml BSA, 0.2mM ATP, 10µg/ml linear double-stranded DNA and trace [γ-^{32}P]ATP.

Concentration: See the product information label for batch-specific information.

References

1. Gottlieb T.M. and Jackson S.P. (1993) The DNA-dependent protein kinase: Requirement for DNA ends and association with Ku antigen. *Cell* **72**, 131–42.
2. Carter, T. *et al.* (1990) A DNA-activated protein kinase from HeLa cell nuclei. *Mol. Cell. Biol.* **10**, 6460–71.
3. Smith, G.C.M. and Jackson, S.P. (1999) The DNA-dependent protein kinase. *Genes Dev.* **13**, 916–34.

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Usage Information

Protocol for Use of DNA-Dependent Protein Kinase:

The following assay protocol may be used to verify the activity of purified DNA-PK. It also may be used as a basis for developing an assay for DNA-PK phosphorylation of protein substrates or for DNA-PK activity in cellular extracts. Dilute or dialyze DNA-PK samples to be assayed into DNA-PK dilution buffer.

Materials to Be Supplied by the User

(Solution compositions are provided below.)

- DNA-PK Peptide Substrate (Cat.# V5671)
- ATP, 10mM (Cat.# P1132)
- [γ-^{32}P]ATP, 3,000Ci/mmol, 10µCi/µl
- acetic acid, 15% and 30%
- Whatman® P-81 phosphocellulose paper
- DNA-PK activation buffer
- 5X DNA-PK reaction buffer
- 10mg/ml BSA

1. Prepare the following reaction as a positive control using a minimum of 10 units of DNA-PK. As additional controls, prepare two reactions lacking either the peptide substrate or the calf thymus DNA.

Component	Volume
5X DNA-PK reaction buffer	10µl
DNA-PK activation buffer	5µl
ATP, 10mM	1µl
DNA-PK Peptide Substrate, 10mg/ml	10µl
[γ-^{32}P]ATP, 3,000Ci/mmol	0.2µl
10mg/ml BSA	0.4µl
DNA-PK (added last; see Note)	10–20u
water to final volume of	50µl
Before adding DNA-PK, pre-incubate the reaction tubes at 30°C for 3 minutes.	

   Note: In the presence of reaction buffer, DNA-PK can autophosphorylate and deactivate itself. Therefore, add the DNA-PK sample to the reaction last (2,3).

2. Incubate for 10 minutes at 30°C; then stop the reaction by adding 20µl of 30% acetic acid.
3. Spot 35µl of the reaction products onto a 2 × 2cm piece of Whatman® P-81 phosphocellulose paper. Allow the reaction products to soak into the paper (approximately 5 seconds).
4. Before the filters dry, wash the filters 5 times for 3–5 minutes each, with swirling, in 15% acetic acid; use 15ml per filter.
5. Using forceps, place the filters on a clean piece of filter paper and allow them to dry completely. Count the samples in a scintillation counter. Reactions using purified DNA-PK should exhibit >tenfold stimulation of ^{32}P incorporation when double-stranded DNA is added compared to control samples with no activation buffer.

Composition of Buffers and Solutions

5X DNA-PK reaction buffer

250mM	HEPES (pH 7.5)
500mM	KCl
50mM	$MgCl_2$
1mM	EGTA
0.5mM	EDTA
5mM	DTT

DNA-PK dilution buffer (1ml)

990µl	1X DNA-PK reaction buffer
10µl	10mg/ml BSA

DNA-PK activation buffer

100µg/ml	calf thymus DNA in 1X TE

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