Product Contents

DNA-Dependent Protein Kinase:

Part No.	Size
V581A	2,500 units

Description: DNA-Dependent Protein Kinase (DNA-PK) consists of a 400kDa 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 product information label for storage recommendations. 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. Performance of this product is guaranteed for six months from date of purchase if stored and handled properly.

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.

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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