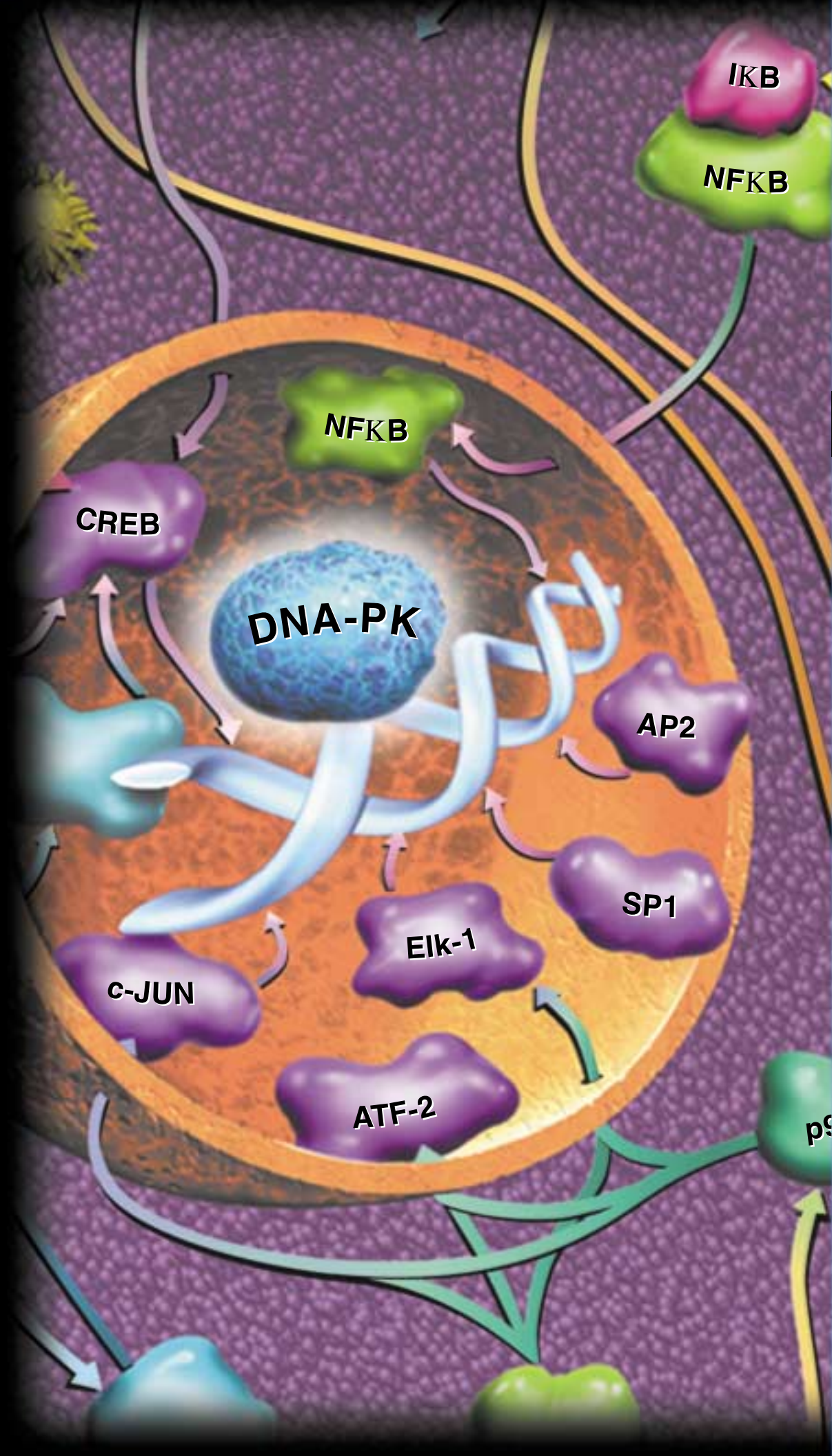


5

CHAPTER

DNA-DEPENDENT PROTEIN KINASE

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SIGNAL TRANSDUCTION RESOURCE

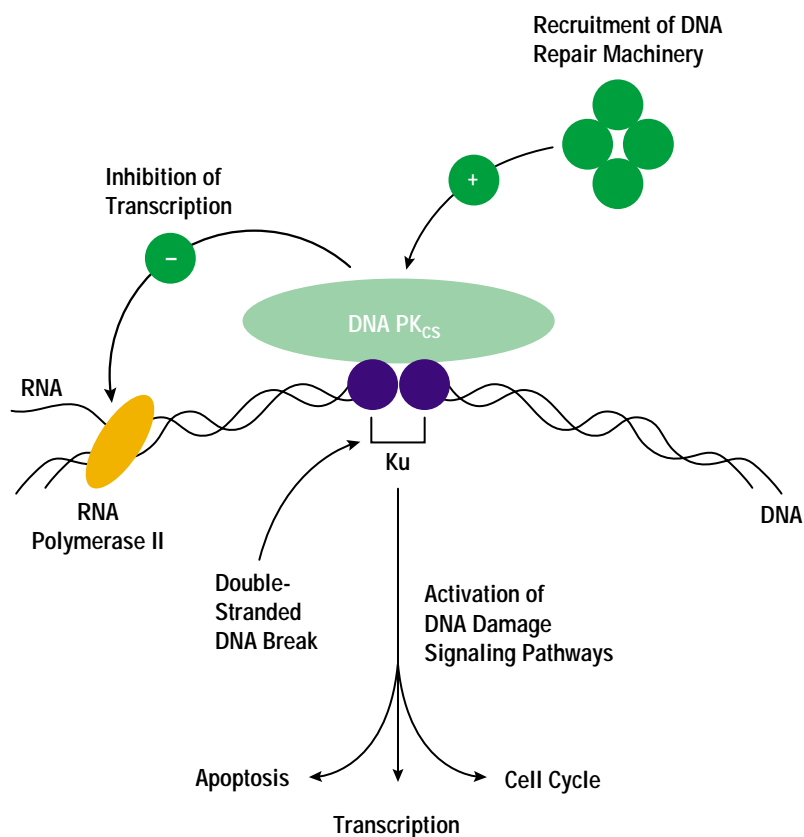


Figure 5.1. Possible mechanisms for DNA-PK acting at the site of a double-stranded break. Activated DNA-PK is depicted promoting DNA repair through several possible mechanisms: 1) recruitment of the DNA double-stranded break repair apparatus by protein-protein interactions and/or by phosphorylation of DNA repair factors; 2) inhibition of other processes that might otherwise interfere with DNA repair, such as transcription; 3) activation of DNA damage signaling events through triggering of protein kinase signaling cascades that impinge ultimately on the apoptotic, transcription and/or cell cycle control machineries.

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DNA-Dependent Protein Kinase

DNA-dependent protein kinase (DNA-PK) is a nuclear serine/threonine kinase that requires double-stranded DNA (dsDNA) for activity (1). DNA-PK phosphorylates target proteins on serine or threonine residues, preferentially, although not exclusively, at the consensus sequence Ser/Thr-Gln (2). The binding of dsDNA to DNA-PK activates the enzyme. Activation by dsDNA brings the enzyme closer to DNA binding proteins, which are substrates for phosphorylation by DNA-PK (3,4).

DNA-PK phosphorylates a large number of substrates (Table 5.1). Mammalian cells deficient in DNA-PK activity cannot undergo V(D)J rearrangement, an essential activity for the generation of immunoglobulin and T-cell receptor hypervariable regions (5). The binding of DNA-PK to double-stranded breaks may protect those sites from nuclease attack or hold them close together to facilitate religation. DNA-PK has been implicated in a variety of other processes including modulation of chromatin structure and telomere maintenance (6).

Table 5.1. Proteins Phosphorylated by DNA-PK in vitro (see reviews 2,6,7).

adenovirus-2 72kDa DNA-binding protein	Oct-1 (POU domain)
bovine papillomavirus	p53
casein	phosvitin
c-Fos	polyoma virus VP1
c-Jun	replication factor A (RPA)
c-Myc	RNA polymerase II (CTD domain)
chicken progesterone receptor	SP1
CTF/NFI	SV40 large T antigen
DNA-PK subunits	tau
hsp 90	TFIID
mdm-2	topoisomerase I and II
microtubule-associated protein	<i>Xenopus</i> histone 2A.X

DNA-PK consists of multiple subunits, including a 470kDa catalytic subunit (DNA-PK_{CS}), and a 150kDa DNA targeting component (Ku antigen) that is a tightly-linked heterodimer (4). The Ku antigen has a high affinity for DNA ends and associates with DNA-PK_{CS}, targeting the catalytic subunit to DNA ends. The Ku antigen is required for the phosphorylation of several proteins, but DNA-PK_{CS} can be stimulated in vitro by certain oligonucleotide sequences without the presence of a DNA-binding subunit. The enzyme is activated by dsDNA but not single-stranded DNA, RNA or DNA/RNA heteroduplexes. Double-stranded DNA with a short, single-stranded end increases DNA-PK activation more than dsDNA alone (8). Since DNA-PK binds dsDNA ends, it is stimulated by linear but not by supercoiled plasmid DNA. The enzyme is activated by a variety of DNA ends, including blunt ends, 5' or 3' overhangs, phosphorylated or nonphosphorylated ends, and closed DNA hairpins. Short, double-stranded oligonucleotides (12bp) can activate the enzyme, but longer duplexes (e.g., 25bp) activate the enzyme at a lower concentration (9). The catalytic subunit is a member of the phosphatidylinositol 3-kinase (PI 3-kinase) family due to an area of homology with the C-terminal ~500 amino acids (10, 11). The kinase activity can be inhibited by classic PI 3-kinase inhibitors like Wortmannin (12,13) and other PI 3-kinase inhibitors like LY 294002, quercitrin and rutin (12).

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SignaTECT® DNA-Dependent Protein Kinase Assay System**TB250**

Publications

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Enzyme Assay System

SignaTECT® Protein Kinase Assay System

Product	Size	Catalog #
SignaTECT® DNA-Dependent Protein Kinase Assay System	96 reactions	V7870

Description: The SignaTECT® DNA-Dependent Protein Kinase (DNA-PK) Assay System^(a) provides an improved method to quantitate DNA-dependent protein kinase activity, both in purified enzyme preparations and in cell nuclear extracts. Promega's SignaTECT® DNA-Dependent Protein Kinase Assay System overcomes the problem of nonspecific substrate binding by using a biotinylated DNA-PK p53-derived peptide substrate in conjunction with Promega's SAM²® Biotin Capture Membrane^(a) (1). The SAM²® Biotin Capture Membrane is a novel streptavidin matrix produced by a proprietary process that results in a high density of streptavidin on the membrane matrix. This streptavidin matrix provides rapid, quantitative capture of biotinylated substrate molecules, based on the strong affinity of biotin for streptavidin ($K_d=10^{-15}M$). The SAM²® Membrane can linearly bind biotinylated substrate in the low nmol/cm² range, depending on the substrate. In addition, the membrane has been optimized for low nonspecific binding.

^(a)U.S. Pat. No. 6,066,462 has been issued to Promega Corporation for quantitation of protein kinase activity.

Reference

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Features

- High Signal-to-Noise Ratios:** The high specificity and strong affinity of the SAM²® Membrane for biotinylated substrates results in lower background and higher signal-to-noise ratios than traditional capture methods (i.e., P-81 phosphocellulose).
- Linear Binding:** Membrane can linearly bind biotinylated substrates up to the nmol/cm² range—allows for kinetic studies.
- Convenient:** SignaTECT® Systems require less “hands-on” manipulation than other assay methods.
- Versatile:** The SAM²® Membrane can be used in a variety of buffer and reaction conditions.

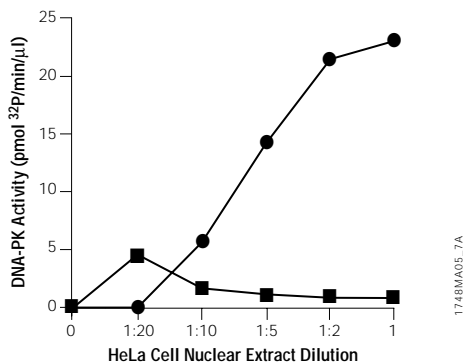


Figure 5.2. DNA-PK activity measured in HeLa cell nuclear extract using Promega's SignaTECT® DNA-PK Assay System. HeLa cell nuclear extracts are prepared by a modification of the method of Dignam *et al.* (1983) *Nucl. Acids Res.* **11**, 1475. Endogenous DNA is removed using a DEAE Sepharose® Fast Flow column. Enzyme activity is quantitated using the SignaTECT® DNA-PK Assay System featuring the SAM²® Biotin Capture Membrane, either in the presence (circles) or absence (squares) of activator (dsDNA).

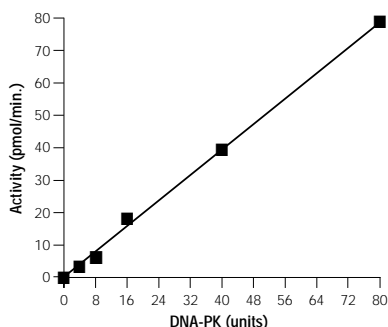


Figure 5.3. Sensitivity and linearity of SignaTECT® DNA-PK Assay System. DNA-PK activity was quantitated over a range of enzyme dilutions using Promega's SignaTECT® DNA-PK Assay System.

Enzymes and Substrates

DNA-Dependent Protein Kinase

Product	Size	Catalog #
DNA-Dependent Protein Kinase	500 units	V5811

Description: DNA-Dependent Protein Kinase (DNA-PK) is a nuclear, serine/threonine protein kinase (1) that requires double-stranded DNA for activity. When activated by DNA, the kinase phosphorylates several DNA-binding substrates in vitro, including the tumor suppressor protein p53, the SV40 large T antigen (2,3) and several transcription factors. Furthermore, efficient phosphorylation of proteins such as SP1 and p53 occurs only when the factor is itself bound to DNA (4-6). DNA-PK is thought to play a role in controlling gene regulation and cell growth.

DNA-PK is isolated from HeLa nuclear extracts as a complex consisting of a 470kDa catalytic subunit and a 155kDa heterodimeric DNA-binding component named Ku, which itself consists of subunits of approximately 85kDa and 70kDa (1,6).

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Usage Note: The enzyme is activated >10 fold by calf thymus DNA (10µg/µl).

DNA-Dependent Protein Kinase Peptide Substrate

Product	Size	Catalog #
DNA-Dependent Protein Kinase Peptide Substrate	1mg	V5671

Description: DNA-Dependent Protein Kinase Peptide Substrate is a peptide substrate for DNA-dependent protein kinase (DNA-PK). The peptide is ready to use in kinase reactions. The sequence of DNA-PK Peptide Substrate is EPPLSQEAFADLWKK. Its molecular weight is 1,759 daltons.

Promega Product Citations

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