

Stressgen

## DnaK Active Recombinant Protein

### Product Specifications

<b>Catalog Number:</b>	SPP-630
<b>Protein Species:</b>	<i>E. coli</i>
<b>Application Notes:</b>	<p><b>ATPase assay:</b>  <i>This protein has tested positive for ATP hydrolysis in an ATPase Assay.</i></p> <p><b>WB Control:</b>  <i>Dilute material into appropriate volume of an SDS containing sample buffer<sup>2</sup> and heat at 100°C for 5 minutes prior to use. On a 15-well minigel system, 50ng of protein per lane should be sufficient when used in a colorimetric Western blot with Dnak Monoclonal Antibody (8E2/2) (SPA-880).</i></p>
<b>Purity:</b>	>95% pure (SDS-PAGE)
<b>Endotoxin Concentration:</b>	<50 EU/mg as determined by Limulus Amebocyte Lysate (LAL) gel clot assay
<b>Format:</b>	40 mM Tris-HCl, pH 7.5, 80 mM NaCl, 0.8 mM DTT, 0.08mM PMSF, 20% glycerol
<b>Storage:</b>	Store at -70°C <i>Shipping conditions may differ from the recommended storage temperature</i>
<b>Related Products:</b>	
SPA-880	Dnak Monoclonal Antibody (8E2/2)
SPP-640	DnaJ Active Recombinant Protein
SPP-650	GrpE Recombinant Protein

### Background:

DnaK is an acidic 70 kDa *Escherichia coli* heat shock protein which belongs to the molecular chaperone class of proteins<sup>1</sup>. The sequence of the *dnaK* gene is highly homologous with eukaryotic Hsp70 proteins. Reversible binding of Hsp70 to unfolded nascent polypeptides, partially denatured proteins and also some native proteins leads to: i) the stabilization of polypeptide chains during *de novo* folding; ii) protection of other proteins from aggregation; iii) reactivation of heat inactivated enzymes; iv) promotion of peptide translocation through membranes; and v) exposure of partially denatured proteins for efficient proteolysis. The N-terminal domains of Hsp70 proteins contain an ATPase active site, and the C-terminal domains contain a peptide binding site. DnaK binds to various aberrant proteins including protein fragments, mutant proteins, unfolded proteins and the native proteins  $\lambda$ P,  $\lambda$ O and  $\sigma$ 32. DnaK's ATPase activity is stimulated by aromatic and aliphatic amino acids.

The *E. coli* DnaK protein can be autophosphorylated and, like all Hsp70 members, exhibits weak ATPase activity<sup>1</sup>. The ADP created during this reaction remains bound to DnaK and can be released by GrpE co-chaperone protein. DnaK's ATPase activity is stimulated up to 50 times by the joint presence of the co-chaperones DnaJ and GrpE heat shock proteins. DnaK changes its conformation in the presence of ATP, triggering release of the protein substrate from the DnaK complex.

Many *in vitro* functions of DnaK protein intensify through cooperation with the *E. coli* molecular chaperones DnaJ and GrpE:

1. Initiation of  $\lambda$ DNA replication. DnaK (in the presence of DnaJ and GrpE proteins) partially disaggregates the proprimosomal complex, allowing DnaB helicase to promote unwinding of double stranded DNA near the *ori*  $\lambda$  sequence.
2. Protection of other proteins from aggregation and involvement in protein folding.
3. Reactivation of heat-inactivated enzymes: *E. coli* RNA polymerase, luciferase, DNA polymerase III from *E. coli*.
4. Activation of native protein for binding to specific DNA sequence: the anti-oncogene p53, P1repA protein, dnaA protein and  $\lambda$ O protein.
5. Activation of mutant proteins.
6. Sequestering  $\sigma$ 32 transcription factor from the RNA polymerase core and probably exposing  $\sigma$ 32 for efficient proteolysis.
7. Facilitating protein translocation through intracellular compartments and protein secretion.

References:

1. Zyllicz, M., *et al.* (1983) PNAS USA, **80**, 6431-6435.
2. Laemmli, U.K., (1970) Nature **227**, 680-685