

CDK1-cyclin B



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P6020S 032120413041

P6020S



200 units 20,000 U/ml Lot: 0321204

RECOMBINANT Store at -70°C Exp: 4/13

Description: CDK1-cyclin B (Cyclin Dependent Kinase 1), also known as Cdc2-cyclin B (Cell division control protein kinase 2) or M-phase promoting factor (MPF), is a member of cyclin-dependent kinases implicated in cell cycle control in eukaryotes. Activation of the CDK1-cyclin B brings the onset of mitosis and is tightly regulated. CDK1-cyclin B is a serine/threonine protein kinase composed of the catalytic subunit CDK1 and its positive regulatory subunit cyclin B (B1 isoform). Binding of CDK1 to cyclin B is essential for activation of the kinase. Phosphorylation

of T161 is required for activation of the CDK1-cyclin B complex and is mediated by the CDK activating kinase (CAK). During G2 phase, CDK1-cyclin B complex is held in an inactive state by phosphorylation of CDK1 at the two negative regulatory sites, T14 and Y15 by CDK1 inhibitory protein kinases, Myt1 and Wee1 respectively. Dephosphorylation of T14 and Y15 by cell division cycle 25 (CDC25) protein phosphatase in late G2 phase activates the CDK1-cyclin B complex and triggers the initiation of mitosis. During expression in insect cells, the recombinant CDK1-cyclin B is activated *in vivo* by endogenous kinase (1-4).

Recognition Determinants: The substrate specificity of CDK1-cyclin B shows an absolute requirement for Pro in the +1 position, a secondary requirement for Arg or Lys at +3, and a preference for basic residues at +2 or +3 positions. The recognition motif for phosphorylation by CDK1-cyclin B is **S/TPXR/K**, frequently with additional basic residues on either side (**K/RSPR/PR/K/H**) (5).

Source: Isolated from *Spodoptera frugiperda* (Sf9) cells infected with recombinant baculovirus carrying human CDK1 and human cyclin B (1,2) (kindly provided by Dr. H. Piwnicka-Worms).

Supplied in: 100 mM NaCl, 50 mM HEPES (pH 7.5 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 0.01% Brij 35 and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer for Protein Kinases (PK).

Reaction Conditions: 1X NEBuffer for PK (NEB #B6022), supplemented with 200 μM ATP (NEB #P0756) and gamma-labeled ATP to a final specific activity of 100-500 μCi/μmol. **Incubate at 30°C.**

1X NEBuffer for PK:
50 mM Tris-HCl
10 mM MgCl₂
0.1 mM EDTA
2 mM DTT
0.01% Brij 35
pH 7.5 @ 25°C

Note that optimal incubation times and enzyme concentrations must be determined empirically for each particular substrate.

Unit Definition: One unit is defined as the amount of CDK1-cyclin B required to catalyze the transfer of 1 pmol of phosphate to CDK Peptide Substrate PKTPKKAKKL-NH₂ (50 μM) in 1 minute at 30°C in a total reaction volume of 30 μl.

Specific Activity: ~1,000,000 units/mg

Molecular Weight: CDK1 (34 kDa), cyclin B (48 kDa). The apparent molecular weight of cyclin B on SDS-PAGE is about 60 kDa.

Quality Control Assays

Protease Activity: After incubation of 100 units of CDK1-cyclin B with a standard mixture of proteins for 2 hours at 30°C, no proteolytic activity could be detected by SDS-PAGE analysis.

Phosphatase Activity: After incubation of 100 units of CDK1-cyclin B with 50 mM *p*-Nitrophenylphosphate for 2 hours at 30°C, no phosphatase activity could be detected by spectrophotometric analysis.

Heat Inactivation: 65°C for 20 minutes.

Quality Assurance: CDK1-cyclin B contains no detectable protease or phosphatase activities.

Notes on Use: Avoid freeze/thaw cycles. Can be stored for 1 week or less at -20°C.

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

References:

1. Parker, L.L. et al. (1991) *The EMBO Journal* 10, 1255–1263.
2. Atherton-Fessler, S. et al. (1993) *Mol. Cell. Biol.* 13, 1675–168
3. McGowan, C.H. and Russel, P. (1993) *The EMBO Journal*, 12, 75–85.
4. Liu, F. et al. (1997) *Mol. Cell. Biol.* 17, 571–583.
5. Songyang, Z. et al. (1994) *Curr. Biol.* 4, 973–982.

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