Store at –80°C

#7544

CDK2/Cyclin A2 Kinase

☑5 µg

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New 02/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant full length human CDK2 kinase and Cyclin A2 protein, supplied as a GST fusion protein.

Background: Cyclin-dependent kinase 2 (p33CDK2) is an important component of the cell cycle machinery. Like p34cdc2, kinase activity is regulated by association with a cyclin subunit, by its phosphorylation state and by association with a CDK inhibitor. Inhibitory phosphorylation occurs on Thr14 and Tyr15 (1). Inhibition of CDK2-cyclin complexes can also be attributed to association with p27Kip1 and p21Waf1/Cip1 (2). Activation of CDK2 complexes requires dephosphorylation of Thr14 and Tyr15 by cdc25 phosphatase and phosphorylation of Thr160 (3), which is mediated by CAK, a complex of CDK7 and cyclin H (4). CDK2/cyclin E kinase activity is important for the G1 to S transition and phosphorylation of the Rb protein. During S-phase, active CDK2/cyclin A complexes predominate and phosphorylate E2F and the active CDK2 complex persists in the nucleus throughout G2 (5).

Source/Purification: The CDK2 and Cyclin A2 proteins were co-expressed using a baculovirus expression system using sf9 cells and recombinant viruses encoding full length human CDK2 (Met1-Leu298) (GenBank Accession No. NM_001798) and Cyclin A2 (Met1-Leu432) (GenBank Accession No. NM_001237), both with an amino-terminal GST tag. The protein complex was purified by one-step affinity chromatography using GSH-agarose.

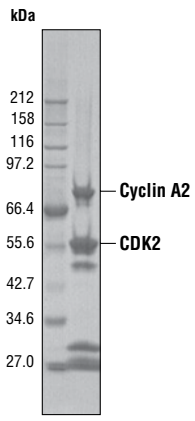

Figure 1. The purity of the GST-CDK2/Cyclin A2 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-CDK2 fusion protein is 58 kDa. The theoretical molecular weight of the GST-Cyclin A2 fusion protein is 78 kDa The purity of the kinase was assessed using SDS-PAGE followed by Coomassie stain [Fig.1]. CDK2/Cyclin A2 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Morgan, D.O. (1995) *Nature* 374, 131–134.

(2) Poon, R.Y. et al. (1996) *J. Biol. Chem.* 271, 13283–13291.

(3) Gu, Y. et al. (1992) *EMBO J.* 11, 3995–4005.

(4) Fesquet, D. et al. (1993) *EMBO J.* 12, 3111–3121.

(5) Morgan, D.O. (1997) *Annu. Rev. Cell Dev. Biol.* 13, 261–291.

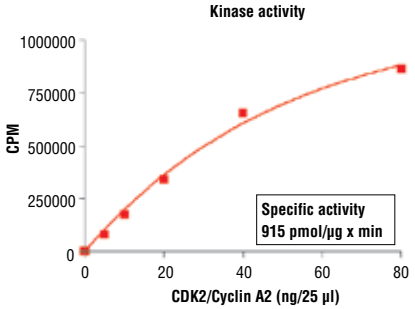

Figure 2. CDK2/Cyclin A2 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: Histone H1 400 ng/µL, and recombinant CDK2/Cyclin A2: variable.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at –80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:
Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Serine/Threonine Kinase Substrate Screening Kit #7400

Protocol for CDK2/Cyclin A2 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Kinase Buffer (10X)**
   50 mM MOPS, pH 7.2
   25 mM β-glycerophosphate
   10 mM EGTA
   4 mM EDTA
   50 mM $MgCl_2$
   0.5 mM DTT
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. Histone H1 (1 µg/µl)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 µM ATP.
2. Dilute [^{32}p] ATP to 0.16 µCi/µl [^{32}p] ATP with 250 µM ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute CDK2/Cyclin A2 protein to 4 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted CDK2/Cyclin A2 kinase solution, 10 µl Histone H1 (1 µg/µl), and 5 µl 0.16 µCi/µl [^{32}p] ATP solution.

Final Assay Conditions
5 mM MOPS, pH 7.2
2.5 mM β-glycerophosphate
1 mM EGTA
4 mM $MgCl_2$
0.05 mM DTT
400 ng/µL Histone H1

6. After 15 minutes terminate reaction by spotting 20 µl of the reaction mixture onto phosphocellulose P81 paper.
7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
9. Count samples in a scintillation counter.

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