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1-866-9 KINASE (54-6273)

| Catalog # | Aliquot Size |
|---|---|
| C29-18G -05 | 5 µg |
| C29-18G -10 | 10 µg |

# CDK2/CyclinE1, Active

Full-length recombinant human protein expressed in Sf9 cells

**Catalog # C29-18G**
*Lot # Q2306-8*

## Product Description

Recombinant full-length human CDK2 and CyclinE1 were co-expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag on both proteins. The gene accession numbers for CDK2 and CyclinE1 are NM_001798 and NM_001238, respectively.

## Gene Aliases

CDK2: p33 (CDK2)
CyclinE1: CCNE1, CCNE

## Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

## Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

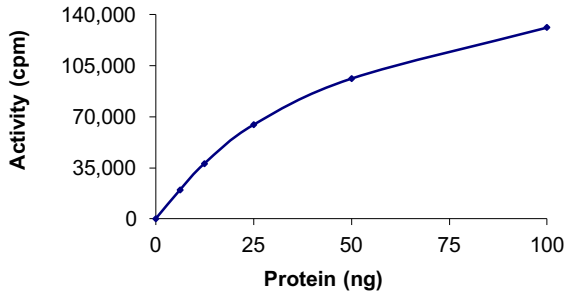
## Scientific Background

CDK2 is a member of the Cyclin-Dependent Kinase family that is ubiquitously expressed. CDK2 is a catalytic subunit of the cyclin-dependent protein kinase complex, whose activity is restricted to the G1-S phase, and essential for cell cycle G1/S phase transition. CDK2 associates with and is regulated by the regulatory subunits of the complex including Cyclin A or E, CDK inhibitor p21Cip1 (CDKN1A) and p27Kip1 (CDKN1B) (1). CDK2 phosphorylates multiple cellular substrates including SMAD3 and FOXO1. Phosphorylation of FOXO1 leads to its inhibition (2).

## References

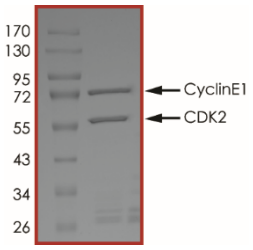

1. Levkau, B. et al: Cleavage of p21(Cip1/Waf1) and p27(Kip1) mediates apoptosis in endothelial cells through activation of Cdk2: role of a caspase cascade. Molec. Cell1: 553-563, 1998.
2. Huang, H. et al: CDK2-dependent phosphorylation of FOXO1 as an apoptotic response to DNA damage. Science314: 294-297, 2006.


## Specific Activity




The specific activity of CDK2/CyclinE1 was determined to be **343 nmol /min/mg** as per activity assay protocol.

## Purity




The purity of CDK2/CyclinE1 was determined to be **>90%** by densitometry, CDK2 approx. MW **58kDa** and CyclinE1 Approx. MW **73kDa**.


# CDK2/CyclinE1, Active

Full-length recombinant human protein expressed in Sf9 cells


| | |
|---|---|
| Catalog # | C29-18G |
| Specific Activity | 343 nmol/min/mg |
| Lot # | Q2306-8 |
| Purity | >90% |
| Concentration | 0.1 µg/µl |
| Stability | 1yr at –70°C from date of shipment |
| Storage & Shipping | Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice. |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# Activity Assay Protocol

## Reaction Components

**Active Kinase** (Catalog #: C29-18G)

Active CDK2/CyclinE1 (0.1μg/μl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active CDK2/CyclinE1 for optimal results).

**Kinase Dilution Buffer III** (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

**Kinase Assay Buffer I** (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**[$^{33}$P]-ATP Assay Cocktail**

Prepare 250μM [$^{33}$P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [$^{33}$P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

**10mM ATP Stock Solution** (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200μl aliquots at –20°C.

**Substrate** (Catalog #: H10-54N)

Histone H1 diluted in distilled $H_2O$ to a final concentration of 1mg/ml.

## Assay Protocol

**Step 1.** Thaw [$^{33}$P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

**Step 2.** Thaw the Active CDK2/CyclinE1, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

**Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active CDK2/CyclinE1 (Catalog #C29-18G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #H10-54N)
- **Component 3.** 5μl distilled $H_2O$ (4°C)

**Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled $H_2O$.

**Step 5.** Initiate the reaction by the addition of 5 μl [$^{33}$P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

**Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

**Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled $H_2O$) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

**Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

**Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

## Calculation of [P$^{33}$]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [$^{33}$P]-ATP / pmoles of ATP (in 5 μl of a 250 μM ATP stock solution, i.e., 1250 pmoles)

## Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of $^{33}$P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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