

This enzyme was purified and extensively validated in a radioactive filter binding assay by a collaborator of Cell Signaling Technology. Cell Signaling Technology is in the process of revalidating this reagent in house using several technologies relevant to the screening community including antibody based detection methods.

Please contact us at [drugdiscovery@cellsignal.com](mailto:drugdiscovery@cellsignal.com) for details.

**Datasheet: CDK2/CycE**

**Description:** Human CDK2 Full Length c-DNA (GenBank accession No. X62071), amino acids M<sub>1</sub> – L<sub>298</sub>.  
Human Cyclin E Full Length c-DNA (GenBank accession No. M73812), amino acids M<sub>1</sub> – A<sub>394</sub>; both N-terminally fused to GST-HIS<sub>6</sub>-Thrombin cleavage site

**Theoretical MW<sub>CDK2</sub>:** 60,222 Da

**Theoretical MW<sub>CyclinE</sub>:** 72,041 Da

**Expression:** Baculovirus infected Sf9 cells (co-expression of both constructs)

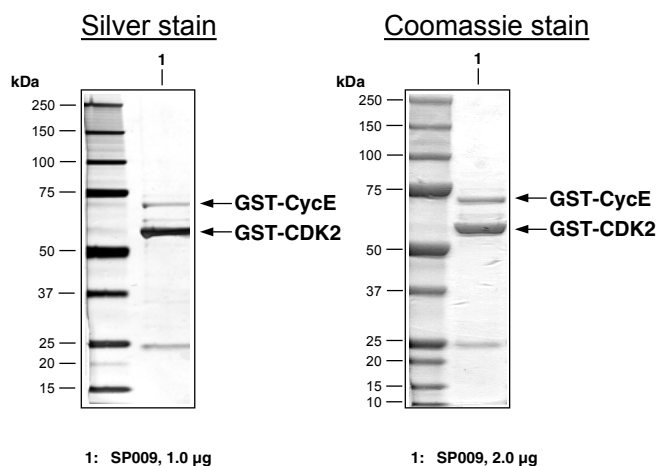
**Purification:** One-step affinity purification using GSH-agarose

**Storage buffer:** 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

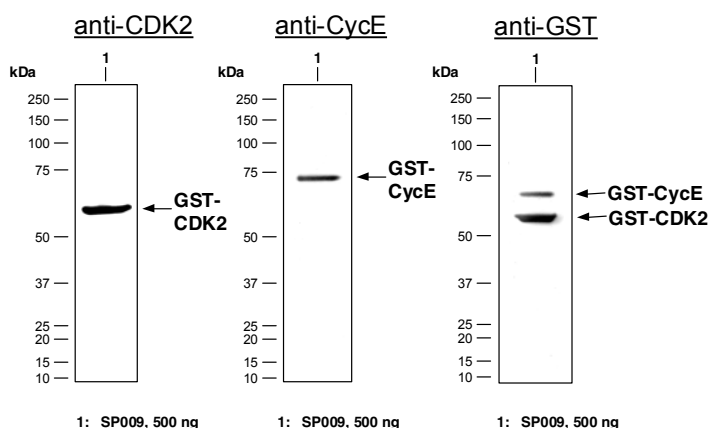
**Storage temperature:** -80°C  
Avoid repeated freeze-thaw cycles!

**Protein concentration:** 0.243 µg/µl (Bradford method using BSA [Sigma: A-7638, Lot# 79H7641] as standard protein)

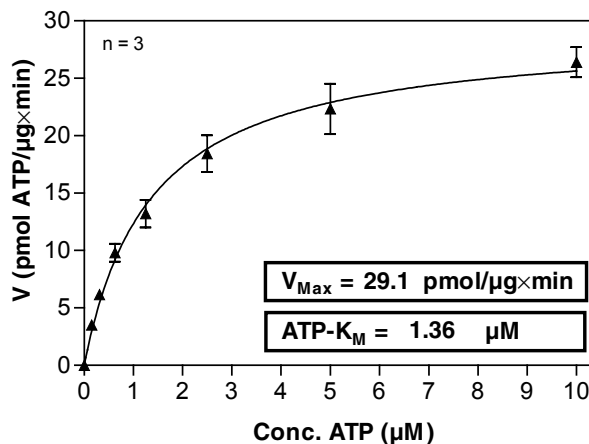
**Purified protein (SDS-PAGE):**



**Purified Protein (Western blot)**



**Kinase activity:**



**Final assay concentrations:**

- 60 mM HEPES-NaOH, pH 7.5
- 3 mM MgCl<sub>2</sub>
- 3 mM MnCl<sub>2</sub>
- 3 µM Na-orthovanadate
- 1.2 mM DTT
- ATP (variable)
- 2.5 µg / 50 µl PEG<sub>20,000</sub>
- Substrate: Histone H1, 2 µg / 50 µl
- Recombinant CDK2/CycE: 100 ng / 50 µl

**Assay: MAFC (Millipore) filter assay**

**Specific activity:** 29 pmol/µg min