Cell Signaling TECHNOLOGY®

#7468

CDK1/CycE

dimeric complex

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 for details.

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

Product No.: 0134-0055-1

Description: Coexpression of human CDK1, amino acids M_1-M_{297} (as in GenBank entry NM_001786)* and human CycE, amino acids M_1-A_{395} (as in GenBank entry M73812)*, N-terminally fused to GST-Thrombin cleavage site

*Sequence may contain documented polymorphisms
Detailed sequence on request

Product identity: CDK1/CycE, Lot 001, was confirmed as CDK1/CycE by specific Western Blotting using anti CDK1 and CycE antibodies

Theoretical $MW_{GST\text{-}CDK1}$: 63,882 Da
Theoretical $MW_{GST\text{-}CycE}$: 72,041 Da

Expression: Baculovirus infected Sf9 cells

Purification: One-step affinity purification using GSH-agarose

Storage buffer: 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20% glycerol

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

Protein concentration: 0.166 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Method for determination of K_m value & Specific activity:

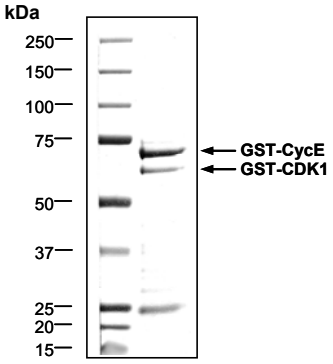
• Assay conditions:

60 mM HEPES-NaOH, pH 7.5
3 mM $MgCl_2$
3 mM $MnCl_2$
3 µM Na-orthovanadate
1.2 mM DTT
2.5 µg / 50 µl $PEG_{20.000}$
ATP (variable)
Substrate: Rb-CTF, 5 µg / 50 µl
Recombinant CDK1/CycE: 200 ng / 50 µl

• Filter binding assay
MSFC membrane (Millipore)

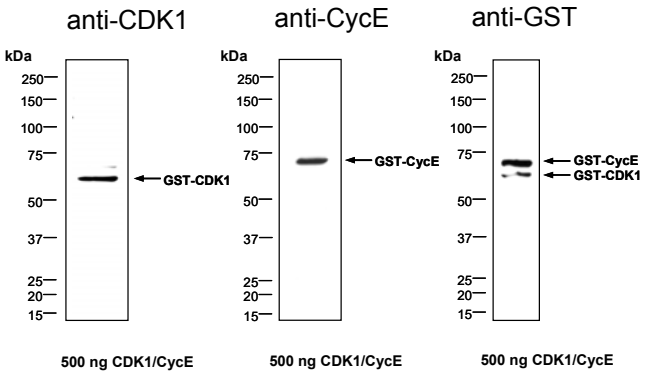
Specific activity: 53 pmol/µg×min

Coomassie stain:



2.0 µg CDK1/CycE

Western blot analysis:



500 ng CDK1/CycE | 500 ng CDK1/CycE | 500 ng CDK1/CycE

Determination of K_m value for ATP: