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1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
C71-10G-05	5 µg
C71-10G-10	10 µg
C71-10G-20	20 µg

CK2α2, Active

Full length recombinant protein expressed in Sf9 cells

Catalog # C71-10G
Lot # E227-3

Product Description

Full length recombinant human CK2α2 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_001896.

Gene Aliases

CSNK2A2; CKII-alpha 2; CK2A2

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.

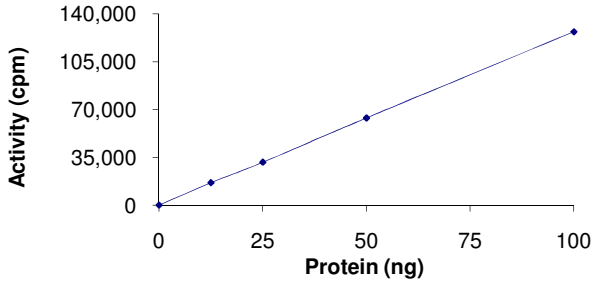
Scientific Background

CK2α2 or casein kinase II alpha 2 is a member of the CK2 family of Ser/Thr protein kinases. CK2a2 plays a fundamental role in cell function and is involved in DNA replication, regulation of basal and inducible transcription, translation and control of metabolism. CK2α2 prefers utilization of acidic proteins such as caseins as substrates. The CK2a2 holoenzyme is a tetramer composed of an alpha chain, an alpha' and two beta chains. The alpha and alpha' chains contain the catalytic site. CK2α2 is also a component of CK2-SPT16-SSRP1 complex comprised of SSRP1, SUPT16H, CSNK2A1, CSNK2A2 and CSNK2B (1). This complex associates following UV irradiation. CK2a2 act as a candidate gene for inherited abnormalities of sperm morphogenesis (2).

References

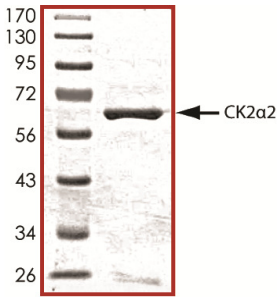
1. Keller, D. M. et.al: A DNA damage-induced p53 serine 392 kinase complex contains CK2, hSpt16, and SSRP1. Molec. Cell 7: 283-292, 2001.
2. Xu, X. et.al: Globozoospermia in mice lacking the casein kinase II alpha-prime catalytic subunit. Nature Genet. 23: 118-121, 1999.

Specific Activity



The specific activity of CK2α2 was determined to be **63 nmol /min/mg** as per activity assay protocol.

Purity



The purity of CK2α2 was determined to be **>95%** by densitometry, approx. MW **~64kDa**.

CK2α2, Active

Full length human recombinant protein expressed in Sf9 cells

Catalog Number	C71-10G
Specific Activity	63 nmol/min/mg
Specific Lot Number	E227-3
Purity	>95%
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 #: C71-10G)

Active CK2α2 (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 CK2α2 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 and 5% glycerol 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 [^{33P}]-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 #: C08-58)

CK2-sub synthetic peptide substrate (RRRADDSDDDDD) 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 CK2α2, 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 CK2α2 (Catalog #C71-10G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #C08-58)
- **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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