

Certificate of Analysis

Casein Kinase 2 Assay Kit

Catalog # 17-132

Lot # JBC1393515

Kit Components

Assay Dilution Buffer I (ADBI), Catalog # 20-108. Two vials, each containing 1 mL of ADBI: 20 mM MOPS, pH 7.2, 25 mM β -glycerol phosphate, 5 mM EGTA, 1 mM sodium orthovanadate, 1 mM dithiothreitol.

Casein Kinase 2 Substrate Peptide, Catalog 12-330, Lot # 0607034940. Two vials, each containing 1 μ mole synthetic peptide (RRRDDDSDDD) in 1 mL of ADBI.

PKA Inhibitor Cocktail, Catalog # 20-114. Two vials, each containing 1 mL of PKA inhibitor cocktail: 2 μ M PKA inhibitor peptide (PKI-[6-22]-NH₂), (Catalog #12-151) in ADBI. An inhibitor which blocks activity of other Serine/Threonine kinases.

Magnesium/ATP Cocktail, Catalog # 20-113. Two vials, each containing 1 mL of Mg²⁺/ATP cocktail: 75 mM magnesium chloride and 500 μ M ATP in ADBI. 90 μ L of the Mg²⁺/ATP cocktail should be added to 10 μ L (100 μ Ci) of [γ -³²P]ATP (3000 Ci/mmol) before starting the assay.

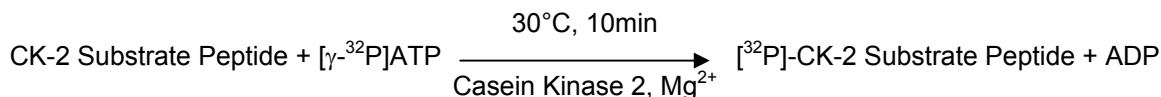
P81 Phosphocellulose Squares, Catalog # 20-134. One pouch containing 200 pre-labeled squares.

Kit Description

Quantity: 200 kinase assays per kit.

Storage and Stability: Stable for 1 year at -20°C from date of shipment.

Use: The assay kit is designed to measure the phosphotransferase activity of Casein Kinase 2 (CK-2) in immunoprecipitates and column fractions. Crude cell lysates may also be used but detergents/biochemicals contained in the cell lysis buffer may inhibit CK-2 activity. Furthermore, although an inhibitor is included with the kit, editors may suggest other unknown kinases found in crude lysates are responsible for CK-2 substrate phosphorylation. The assay kit is based on phosphorylation of a specific substrate (CK-2 substrate peptide) using the transfer of the gamma-phosphate of [γ -³²P]ATP by CK-2 kinase. The phosphorylated substrate is then separated from the residual [γ -³²P]ATP using P81 phosphocellulose paper and quantitated by using a scintillation counter. The assay is linear for incubation times of up to 30 minutes and incorporation of up to 20% of total ATP. Further incubation or incorporation may not be linear and may therefore not be a true indication of CK-2 activity in the sample extract. The enzyme assay is rapid, convenient and fairly specific for CK-2. Each kit contains sufficient reagents for 200 individual CK-2 assays.



**FOR RESEARCH USE ONLY. NOT RECOMMENDED OR INTENDED
FOR DIAGNOSIS OF DISEASE IN HUMANS OR ANIMALS.
DO NOT USE IN HUMANS OR IN ANIMALS**

Other components required but not included as part of kit are:

- **Enzyme Preparation containing Casein Kinase 2:** 10-200 µg protein/immunoprecipitate diluted into assay dilution buffer or 10-100 ng of purified active Casein Kinase 2 (Catalog # 14-197).
- vortex mixer
- Plexiglas shielding
- incubating water bath
- timer
- Trichloroacetic Acid (TCA)
- variable volume (5-200 µL) pipet + tips
- phosphoric acid
- scintillation vials
- scintillation fluid
- scintillation counter
- [γ -³²P]ATP – 3000 Ci/mmol, obtained from Perkin-Elmer, Cat. # BLU002A.

Safety Warnings and Precautions: The Casein Kinase 2 assay kit is designed for research use only and not recommended for internal use in humans or animals. Since the kit involves the use of radioactive [γ -³²P]ATP, please follow your institutional instructions for handling, use, storage and disposal of radioactive materials. All chemicals should be considered potentially hazardous and principles of good laboratory practice should be followed.

Casein Kinase 2 Assay Kit Procedures

Assay Protocol Summary:

- * Perform all pre-incubation reactions over an ice bath.
- 1. Rapidly thaw the kit components, mix by vortexing and place on ice before proceeding with the assay. The assay components can be refrozen and stored at -20°C for extended periods of time.
- 2. Add 10 µL of ADBI to a microcentrifuge tube.
- 3. Add 10 µL of the substrate peptide (200µM final concentration).
- 4. Add 10 µL of PKA inhibitor cocktail or ADBI.
- 5. Add 10 µL of Casein Kinase 2 (10-100ng purified enzyme/assay or 10-200 µg protein/immunoprecipitate).
- 6. Add 10 µL of the diluted [γ -³²P]ATP.
- 7. Incubate and agitate for 10 minutes at 30°C.
- 8. Stop the reaction by adding 20µl of 40% TCA to each microcentrifuge tube.
- 9. Transfer 25 µL aliquot on numbered P81 paper square and allow the radiolabelled substrate to bind to the paper for 30 seconds.
- 10. Immerse the paper in 0.75% phosphoric acid, mix gently on a rotator. Use 40 mL in a 50 mL conical tube.
- 11. Wash six times with 0.75% phosphoric acid for 1 minute per wash, to reduce background. Dispose each wash in accordance with local radioisotope regulations.
- 12. Wash the squares in 20 mL of acetone for 1 minute.
- 13. Allow to dry, transfer to a scintillation vial and add scintillation cocktail.
- 14. Read in scintillation counter. Compare CPM of enzyme samples to CPM of control samples that contain no enzyme (background control).
- * Suitable blanks should always be performed to correct for non-specific binding of [γ -³²P]ATP and its breakdown products to the phosphocellulose paper. Controls for endogenous phosphorylation of proteins in the sample extract can be performed by substituting assay dilution buffer for substrate cocktail.

Technical Note: Allow the radiolabeled substrate to bind to the filter paper for 30 seconds before immersing the paper into a 50 mL conical tube containing 40 mL 0.75% phosphoric acid. Gently shake the assay squares for 5 minutes on a rotator. Discard the wash in a liquid radioisotope waste container, (dispose of per institutional regulations) and repeat the wash step twice. Wash the squares in 20 mL of acetone for 5 minutes. Drain and add scintillation cocktail.

Casein Kinase 2 Assay Data: Casein Kinase 2 activity was measured using CK-2 substrate peptide as a kinase substrate and a separate inhibitor cocktail which blocks the activity of other serine/threonine kinases such as protein kinase A. Test results are shown to the right:

CK-2 Enzyme	Substrate Peptide	Mean CPM	Comments
50 ng (B)	None	4,238	Enzyme Background
None	200 μM	3,882	Substrate Background
50 ng (A)	200 μM	811,997	CK-2 Activity

Determination of Casein Kinase 2 Activity:

Determine the specific radioactivity of the Mg²⁺/cold ATP-hot ATP mixture. Assume that the amount of hot ATP is negligible. In the above experiment, 1 μL of the ATP solution gave 1.34 X 10⁶ CPM, therefore 10 μL would give 1.34 X 10⁷ CPM = 5000 pmol ATP (500 μmoles/liter x 1x10⁻⁵ liters).

$$\begin{aligned}
 &1.34 \times 10^7 \text{ CPM} / 5000 \text{ pmol ATP} \\
 &= 2,682 \text{ CPM/pmol ATP} \\
 &= \text{Specific Radioactivity (S.R.)}
 \end{aligned}$$

The [³²P] incorporated into the substrate is quantitatively measured by its binding to the phosphocellulose paper. In the presence of sample extract, the [³²P] counted on the paper is the sum of non-specific [³²P]ATP binding, specific binding of phosphorylated substrate and binding of phosphorylated endogenous proteins in the sample extracts (A). In the absence of substrate the [³²P] counted on the papers is due to non-specific binding of [³²P]ATP and its breakdown products and binding of phosphorylated endogenous proteins in the sample extracts (B). Therefore, the [³²P] incorporated into the substrate is obtained from (A-B).

Since only 25μl of the incubation mixture was spotted onto the P81 paper out of a total volume of 70 μL, the total [³²P] incorporated into the substrate is given by (A-B) X 2.8.

$$\frac{(A-B) \times 2.8}{\text{S.R.} \times 10\text{min}} = \text{pmol phosphate incorporated into CK-2 Sub. Peptide/minute}$$

In the above example: $\frac{(811,997-4,238) \times 2.8}{2,682 \times 10 \text{ minutes}}$

= 84.3 pmol phosphate incorporated into CK-2 Substrate Peptide/min/50 ng of CK-2

= 1.687 pmol phosphate incorporated into CK-2 Substrate Peptide/min/ng of CK-2