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Certificate of Analysis

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Casein Kinase-2 Assay Kit

Catalog # 17-132
Lot # 18642

Kit Components

Assay Dilution Buffer (ADB), Catalog # 20-108. Two vials, each containing **1ml** of assay dilution buffer: 20mM MOPS, pH 7.2, 25mM β-glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol.

Casein Kinase-2 Substrate Peptide, Catalog 12-330, Lot # 18643. Two vials, each containing **1ml** of 1mM Casein Kinase-2 (CK-2) Substrate Peptide in ADB.

Inhibitor Cocktail, Catalog # 20-114, Lot # 17653. Two vials, each containing **1ml** of inhibitor cocktail in ADB (2µM protein kinase A inhibitor peptide [PKI] [Cat. #12-151]). An inhibitor which blocks activity of other Serine/Threonine kinases.

Magnesium/ATP Cocktail, Catalog # 20-113. Two vials, each containing **1ml** of Mg^{2+}/ATP cocktail: 75mM magnesium chloride and 500µM ATP in ADB. 90µl of the Mg^{2+}/ATP cocktail should be added to 10µl (100µCi) of the [γ-^{32}P]ATP (~3000 Ci/mmol) before starting the assay.

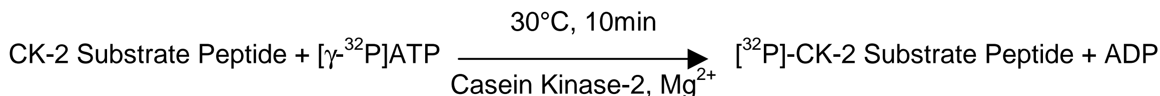
P81 Phosphocellulose Squares, Catalog # 20-134 One pouch containing 200 pre-labeled squares.

Kit Description

Quantity: 200 kinase assays per kit.

Storage and Stability: Stable for 2 years 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-^{32}P]ATP by CK-2 kinase. The phosphorylated substrate is then separated from the residual [γ-^{32}P]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:** 10-200µg protein/immunoprecipitate diluted into assay dilution buffer or 50-500ng of purified Casein Kinase (Catalog # 14-197).
- vortex mixer
- Plexiglas shielding
- incubating water bath
- timer
- Trichloroacetic Acid (TCA)
- variable volume (5-200µl) pipet + tips
- phosphoric acid
- scintillation vials
- scintillation fluid
- scintillation counter
- [γ-^{32}P]ATP - ~3000 Ci/mmol, obtained from DuPont-New England Nuclear.

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-^{32}P]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 10µl of ADB to a microcentrifuge tube.
3. Add 10µl of the substrate peptide (200µM final concentration with inhibitor; 250µM without inhibitor).
4. Add 10µl of the inhibitor cocktail or ADB.
5. Add 10µl of Casein Kinase-2 (50-500ng purified enzyme/assay or 10-200µg protein/immunoprecipitate).
6. Add 10µl of the diluted [γ-^{32}P]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 25µl 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 40ml in a 50ml conical tube.
11. Wash 2-10 times with phosphoric acid to reduce background. Dispose each wash in accordance with local radioisotope regulations.
12. Wash one time with acetone, 20ml per tube.
13. Allow papers 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-^{32}P]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.

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

CK-2 Enzyme	Substrate Peptide	Mean CPM	Comments
200ng (B)	None	9,447	Endogenous Phosphorylation
200ng (A)	200µM	577,115	CK-2 Activity

Determination of Casein Kinase-2 Activity:

Determine the specific radioactivity of the Mg^{2+}/cold ATP-hot ATP mixture. Assume that the amount of hot ATP is negligible. In the above experiment, 5µl of the ATP solution gave 6.30 X 10^6CPM, therefore 10µl would give 12.6 X 10^6CPM = 5000pmol ATP (500µmoles/liter x $1x10^{-5}$ liters).

12.6 X 10^6CPM/5000pmol ATP
= 2520 CPM/pmol ATP
= Specific Radioactivity (S.R.)

The [^{32}P] incorporated into the substrate is quantitatively measured by its binding to the phosphocellulose paper. In the presence of sample extract, the [^{32}P] counted on the paper is the sum of non-specific [^{32}P]ATP binding, specific binding of phosphorylated substrate and binding of phosphorylated endogenous proteins in the sample extracts (A). In the absence of substrate the [^{32}P] counted on the papers is due to non-specific binding of [^{32}P]ATP and its breakdown products and binding of phosphorylated endogenous proteins in the sample extracts (B). Therefore, the [^{32}P] 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 70µl, the total [^{32}P] incorporated into the substrate is given by (A-B) X 2.8.

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

In the above example:

$$\frac{(577{,}115-9{,}447) \times 2.8}{2520 \times 10 \text{ minutes}}$$

= 63pmol phosphate incorporated into CK-2 Substrate Peptide/min/200ng of CK-2

= 0.32pmol phosphate incorporated into CK-2 Substrate Peptide/min/ng of CK-2