

Casein Kinase II Assay Kit

Catalog # 17-132

Lot # 16134

Kit Components

Assay Dilution Buffer (ADB), Catalog # 20-108

Two vials, each containing **1.0ml** of assay dilution buffer: 20mM MOPS, pH 7.2, 25mM β -glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol.

Casein Kinase II Substrate Peptide, Catalog 12-330, Lot # 16131

Two vials, each containing **1.0ml** of 1mM Casein Kinase II (CK II) Substrate Peptide in ADB.

Inhibitor Cocktail, Catalog # 20-114, Lot # 15545

Two vials, each containing **1.0ml** of inhibitor cocktail in assay dilution buffer (2 μ M 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, Lot # 15953

Two vials, each containing **1.0ml** of Mg²⁺/ATP cocktail: 75mM magnesium chloride and 500 μ M ATP in ADB. 90 μ l of the Mg²⁺/ATP cocktail should be added to 100 μ Ci (10 μ l) of the [γ -³²P]ATP (~3000 Ci/mmol) before starting the assay.

P81 Phosphocellulose Squares, Catalog # 20-134

One pouch containing 200 pre-labeled squares.

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

- **Enzyme Preparation containing Casein Kinase II:** 10-200 μ g 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 (5-200 μ l) pipet + tips
- phosphoric acid
- scintillation vials
- scintillation fluid
- scintillation counter
- [γ -³²P]ATP - ~3000 Ci/mmol, obtained from DuPont-New England Nuclear.

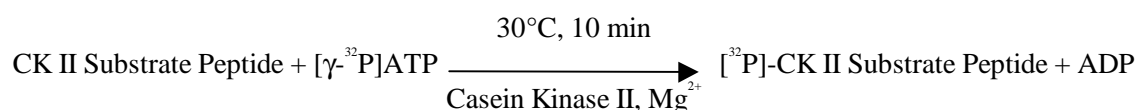
Lot Analysis

Quantity: 200 kinase assays per kit.

Handling and Use

Storage and Stability: 6 months at -20°C.

Use: The assay kit is designed to measure the phosphotransferase activity of Casein Kinase II (CK-II) in immunoprecipitates and column fractions. Crude cell lysates may also be used but detergents/biochemicals contained in the cell lysis buffer may inhibit CK-II activity. Furthermore, although an inhibitor is included with the kit, editors may suggest other unknown kinases found in crude lysates are responsible for CK-II substrate phosphorylation. The assay kit is based on phosphorylation of a specific substrate (CK-II substrate peptide) using the transfer of the gamma-phosphate of [γ - 32 P]ATP by CK-II 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-II activity in the sample extract. The enzyme assay is rapid, convenient and fairly specific for CK-II. Each kit contains sufficient reagents for 200 individual CK-II assays.



Safety Warnings and Precautions: The Casein Kinase II 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.

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DO NOT USE IN HUMANS OR IN ANIMALS**

Casein Kinase II Assay Kit Procedures

The kit components should be rapidly thawed, mixed by vortexing and stored on ice before proceeding with the assay. Do not use extended thawing time. In particular, ADB and Magnesium/ATP cocktail must be mixed completely before beginning the assay. The assay components can be refrozen at -20°C for extended periods. Perform all preincubation reactions at 1°C over an ice bath. The kinase assay may also be performed at room temperature but will not give as linear results.

Aliquot 10µl of substrate cocktail, 10µl of inhibitor cocktail and 10µl of enzyme preparation into the bottom of a microcentrifuge tube. Start the reaction by adding 10µl of the Mg²⁺/ATP cocktail containing [γ -³²P]ATP, vortex gently and incubate the microcentrifuge tube at 30°C for 10 minutes. Stop the reaction by adding 20µl of 40% TCA to each sample. Remove 25µl of the reaction mixture and pipet slowly onto the center of a P81 phosphocellulose paper square. Allow the radiolabelled substrate to bind to the filter paper for 30 seconds before immersing the paper into a beaker containing 0.75% phosphoric acid. Wash the papers thoroughly in the beaker with up to 10 rinses of 0.75% phosphoric acid. Do not wash more than 30 papers per beaker. Dispose of each rinse according to local radioisotope regulations. After washing, add acetone and wash for 2 minutes, remove the paper with forceps and stuff the paper into a 5ml scintillation vial. Add scintillation cocktail and quantitate the bound radioactivity on the paper in a scintillation counter for 1 minute. Alternatively, assay squares can be washed by adding to a 50ml conical tube containing 40ml 0.75% phosphoric acid. Gently shake the assay squares for 5 minutes on a rotator. Discard the wash in a liquid radioisotope waste container and repeat the wash step twice. Wash the squares in 20ml of acetone for 5 minutes. Drain and add scintillation cocktail.

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.

Assay Protocol Summary:

1. Add 10µl of the substrate cocktail to a microcentrifuge tube.
2. Add 10µl of the inhibitor cocktail or ADB to a microcentrifuge tube.
3. Add 10µl of Casein Kinase II (50-500ng purified enzyme/assay or 10-200µg protein/immunoprecipitate).
4. Add 10µl of the diluted [γ -³²P]ATP. 90µl of the Mg²⁺/ATP cocktail should be added to 100µCi (10µl) of the [γ -³²P]ATP (~3000 Ci/mmol) before starting the assay.
5. Add ADB to bring volume up to 50µl.
6. Incubate and agitate for 10 minutes at 30°C.
7. Stop the reaction by adding 20µl of 40% TCA to each microcentrifuge tube.
8. Blot 25µl aliquot on numbered P81 paper square.
9. Wash the assay squares with up to 10 rinses of 0.75% phosphoric acid.
10. Transfer assay squares to a scintillation vial and add scintillation cocktail.
11. Read in scintillation counter. Compare CPM of enzyme samples to CPM of control samples that contain no enzyme (background control).

Casein Kinase II Assay Data: Casein Kinase II activity was measured using this kit. This kit uses CK-II 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 below:

CK II Enzyme	Inhibitor	Substrate	Mean CPM	Comments
None (B)	None	CK II Sub. Peptide	18,712	Background
200ng (C)	None	None	19,564	Endogenous Phosphorylation
200ng (A)	PKA	CK II Sub. Peptide	2,826,614	CK II Activity
200ng	None	CK II Sub. Peptide	3,856,940	Total Kinase Activity

Determination of Casein Kinase II 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, 5 μ l of the ATP solution gave 6.7 X 10⁶CPM, therefore 10 μ l would give 13.4 X 10⁶CPM = 5000pmoles ATP (500 μ moles/liter x 1x10⁻⁵ liters).

$$13.4 \times 10^6 \text{ CPM} / 5000 \text{ pmoles ATP} \\
= 2680 \text{ CPM/pmoles ATP} \\
= \text{Specific Radioactivity (S.R.)}$$

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 enzyme, the [³²P] counted on the paper is due to non-specific binding of [³²P]ATP and its breakdown products (B).

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 (C).

Therefore, the [³²P] incorporated into the substrate is obtained from (A-B) - (C-B).

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

$$\frac{[(A-B) - (C-B)] \times 2.8}{\text{S.R.} \times 10 \text{ min}} = \text{pmoles phosphate incorporated into CK-II Sub. Peptide/minute}$$

$$\text{In the above example: } \frac{[(2,826,614 - 18,712) - (19,564 - 18,712)] \times 2.8}{2680 \times 10 \text{ minutes}}$$

$$= 293 \text{ pmoles phosphate incorporated into CK-II Substrate Peptide/min/200ng of CK-II}$$

$$= 1.47 \text{ pmoles phosphate incorporated into CK-II Substrate Peptide/ng of CK-II}$$