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## Certificate of Analysis

### Casein Kinase 2 Assay Kit

Catalog # 17-132

Lot # JBC1361050

#### Kit Components

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

**Casein Kinase 2 Substrate Peptide**, Catalog 12-330, Lot # 0607034940. Two vials, each containing 1 $\mu$ mol synthetic peptide (RRRDDDSDDD) in 1ml of ADBI.

**PKA Inhibitor Cocktail**, Catalog # 20-114. Two vials, each containing 1ml of PKA inhibitor cocktail: 2 $\mu$ M PKA inhibitor peptide (PKI-[6-22]-NH<sub>2</sub>), (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 1ml of Mg<sup>2+</sup>/ATP cocktail: 75mM magnesium chloride and 500 $\mu$ M ATP in ADBI. 90 $\mu$ l of the Mg<sup>2+</sup>/ATP cocktail should be added to 10 $\mu$ l (100 $\mu$ Ci) of [ $\gamma$ -<sup>32</sup>P]ATP (3000Ci/mmol) before starting the assay.

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

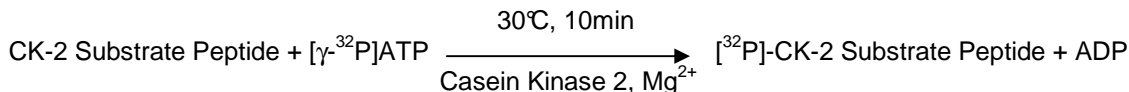
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#### Kit Description

**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 [ $\gamma$ -<sup>32</sup>P]ATP by CK-2 kinase. The phosphorylated substrate is then separated from the residual [ $\gamma$ -<sup>32</sup>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 10-100ng of purified active Casein Kinase 2 (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
- [ $\gamma$ -<sup>32</sup>P]ATP - 3000Ci/mmol, obtained from Perkin-Elmer, Cat. # BLU002A.

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**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 [ $\gamma$ -<sup>32</sup>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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### 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 ADBI to a microcentrifuge tube.
- 3. Add 10µl of the substrate peptide (200µM final concentration).
- 4. Add 10µl of PKA inhibitor cocktail or ADBI.
- 5. Add 10µl of Casein Kinase 2 (10-100ng purified enzyme/assay or 10-200µg protein/immunoprecipitate).
- 6. Add 10µl of the diluted [ $\gamma$ -<sup>32</sup>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 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 20ml 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 [ $\gamma$ -<sup>32</sup>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.

**Technical Note:** Allow the radiolabeled substrate to bind to the filter paper for 30 seconds before immersing the paper into 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, (dispose of per institutional regulations) and repeat the wash step twice. Wash the squares in 20ml 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
50ng (B)	None	4,238	Enzyme Background
None	200µM	3,882	Substrate Background
50ng (A)	200µM	811,997	CK-2 Activity

**Determination of Casein Kinase 2 Activity:**

Determine the specific radioactivity of the Mg<sup>2+</sup>/cold ATP-hot ATP mixture. Assume that the amount of hot ATP is negligible. In the above experiment, 1µl of the ATP solution gave 1.34 X 10<sup>6</sup> CPM, therefore 10µl would give 1.34 X 10<sup>7</sup> CPM = 5000pmol ATP (500µmoles/liter x 1x10<sup>-5</sup> 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 [<sup>32</sup>P] incorporated into the substrate is quantitatively measured by its binding to the phosphocellulose paper. In the presence of sample extract, the [<sup>32</sup>P] counted on the paper is the sum of non-specific [<sup>32</sup>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 [<sup>32</sup>P] counted on the papers is due to non-specific binding of [<sup>32</sup>P]ATP and its breakdown products and binding of phosphorylated endogenous proteins in the sample extracts (B). Therefore, the [<sup>32</sup>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 [<sup>32</sup>P] incorporated into the substrate is given by (A-B) X 2.8.

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

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

$$= 84.3\text{pmol phosphate incorporated into CK-2 Substrate Peptide/min}/50\text{ng of CK-2}$$

$$= 1.687\text{pmol phosphate incorporated into CK-2 Substrate Peptide/min}/\text{ng of CK-2}$$