





# Choline Kinase (ChoK) Activity Assay Kit (Fluorometric)

(Catalog # K476-100; 100 assays, Store kit at -20°C)

rev 07/20

#### I. Introduction:

Choline Kinase (E.C. 2.7.1.32) is an enzyme that catalyzes the first reaction in the CDP-Choline pathway for the synthesis of Phosphatidylcholine, a membrane phospholipid. This transferase utilizes  $Mg^{2+}$  as a cofactor in order to transfer a phosphate moiety from ATP to Choline molecules forming Phosphocholine. In humans, there are two isozymes, Choline Kinase A and B. Both proteins share more than 60% in homology. Choline Kinase plays pivotal role in different human diseases. Elevated Choline Kinase A expression has been found in breast, lung, colorectal, prostate and bladder tumor tissues whereas Choline Kinase B is suggested to be involved in muscular dystrophy. In **BioVison's Choline Kinase Activity Assay Kit**, choline is phosphorylated by Choline Kinase producing a series of intermediates, which react with the fluorometric probe generating a strong flurorescence signal (Ex/Em = 535/587 nm). The signal is directly proportional to Choline Kinase activity present in the biological sample. The assay is fast, sensitive, reproducible and is suitable for measuring Choline Kinase activity in different cells and other biological samples This kit can detect as low as 0.5  $\mu$ M of Choline Kinase activity.

Choline + ATP Choline Kinase Phosphocholine + ADP Probe Fluorescence (Ex/Em = 535/587 nm)

## II. Applications:

· Measurement of Choline Kinase activity in different cell lines and tissues

## III. Sample Type:

- Purified Protein
- · Cell/tissue lysates: kidney, liver

#### IV. Kit Contents:

Components	K476-100	Cap Code	Part Number
Assay Buffer	50 ml	NM	K476-100-1
Choline Substrate	1 vial	Green	K476-100-2
Detection Mix I	1 vial	Purple	K476-100-3
Detection Mix II	1 vial	Red	K476-100-4
ATP	1 vial	Orange	K476-100-5
Probe	0.4 ml	Blue	K476-100-6
ADP Standard (1 µmole)	1 vial	Yellow	K476-100-7
Human ChoK	10 μΙ	Brown	K476-100-8

# V. User Supplied Reagents and Equipment:

- 96-well white flat-bottom fluorescent plate
- · Multi-well spectrophotometer
- 10K Spin Column (BV Cat. # 1997 or similar)

## VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. The kit components are stable for one year when stored as recommended. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- Assay Buffer: Ready to use as supplied. Bring it to room temperature (RT) before use. Store at 4°C.
- Choline Substrate: Reconstitute with 1.25 ml of dH₂O to generate Choline Substrate solution. Keep on ice while in use. Store at -20°C.
- Detection Mix I and Detection Mix II: Reconstitute each vial with 220 μI of Assay Buffer separately. Pipette up and down to dissolve completely. Store at -20°C. Keep on ice while in use. Stable for 2 months at -20°C.
- ATP: Reconstitute with 220 µl of dH<sub>2</sub>O to generate ATP stock solution. Divide into aliquots and store at -20°C. Keep on ice while in use.
- Probe: Thaw probe at RT before use. Store at -20°C. Use within two months.
- ADP Standard (1 μmole): Reconstitute with 1 ml of dH<sub>2</sub>O to generate 1 mM stock ADP Standard. Divide into aliquots and store at -20°C. Keep on ice while in use.
- Human ChoK: Ready to use. Store at -20°C. Use within two months.

## VII. Choline Kinase Activity Assay Protocol:

1. Sample Preparation: Homogenize cells (1 x 10<sup>6</sup>-10<sup>7</sup>) with 200 μl of cold Assay Buffer or 100 mg of tissue (e.g. liver or kidney tissue) in 400 μl of Assay Buffer for 5 min. After homogenization, keep on ice for 15 min. Centrifuge at 10,000 x g for 20 min. and transfer supernatant to a new tube. To reduce endogenous background, transfer 50 μl of the supernatant to a 10K Spin Column (BioVision Cat. # 1997 or similar) and add 450 μl of Assay Buffer. Centrifuge at 10,000 x g for 20 min. Remove eluate, add 500 μl of the Assay Buffer to the retentate and centrifuge again. Repeat the filtration step for 3 times. After filtration is complete, collect the retentate and measure its volume. Dilute it by 20-100 folds using the Assay Buffer. Add 5-10 μl of the diluted supernatant to a well. Positive Control: Dilute Human ChoK 100-fold (eg. 2 μl in 198 μl of Assay Buffer). Add 5 μl of the diluted Human ChoK to a well. Adjust the volume to 50 μl/well with Assay Buffer.

## Notes:

- a. For Unknown Samples, we recommend doing pilot experiments and testing several doses to ensure the readings are within the Standard Curve range.
- b. For samples exhibiting significant background, prepare sample well(s) designated as Sample Background Control(s).

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- 2. ADP Standard: Dilute the 1 mM stock ADP Standard to 10 μM ADP Standard by adding 10 μl of 1 mM stock ADP Standard into 990 μl of water. Add 0, 5, 10, 15, 20 μl of 10 μM ADP Standard into the desired wells of a 96-well white flat-bottom plate to generate 0, 50, 100, 150, 200 pmole ADP Standard/well. Adjust the volume of all Standard wells to 50 μl/well with Assay Buffer.
- 3. Reaction Mix: Mix enough reagents for the number of assays to be performed. Prepare the following Reaction Mix.

	Reaction Mix	Sample Background Mix
Assay Buffer	33 µl	43 µl
Choline Substrate solution	10 µl	µl
Detection Mix I	2 µl	2 µl
Detection Mix II	2 µl	2 µl
ATP	2 µl	2 µl
*Probe	1 µl	1 µl

\*To reduce background, mix all components but probe prior to its addition. Incubate for 2 min and then add indicated Probe volume to the mixture. Mix well, and add 50 µl Reaction Mix to the wells containing Standards, Samples and Positive Control. Add 50 µl Sample Background Mix to the samples designated as Sample Background Control(s).

4. Measurement: Measure fluorescence at Ex/Em = 535/587 nm in kinetic mode at 25°C for 30 min.

**Note:** Incubation time depends on Choline Kinase enzymatic activity in samples. Long incubation time may be required for samples having low Choline Kinase activity.

5. Calculation: Plot the ADP Standard Curve. If the sample background is significant, subtract the Sample Background Control reading from its paired sample reading. Calculate the Choline Kinase activity of the test sample: ΔRFU = RFU<sub>2</sub> – RFU<sub>1</sub>. Apply the ΔRFU to the ADP Standard Curve to get B pmole of ADP generated during the reaction time (Δt = t<sub>2</sub> – t<sub>1</sub>).

## Specific Activity = B x D / ( $\Delta t$ x V) = pmol/min/ml ( $\mu$ U/ml)

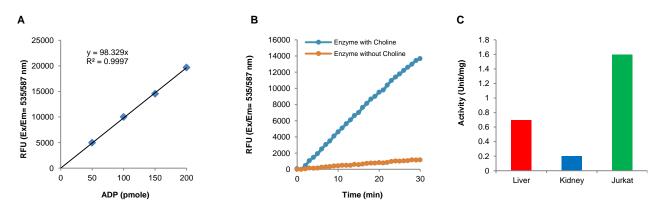
Where: B is ADP from the Standard Curve (pmol)

Δt is Reaction time (min)

V is sample volume added into the reaction well (ml)

**D** is Dilution factor

Unit Definition: One unit is 1 µmole of ADP generated by Choline Kinase per min at pH 8 and 25°C.



Figures. A. ADP Standard Curve. B. Kinetic reaction rate of ChoK with and without choline substrate in the assay. C. Activity of ChoK determined by the assay in different samples.

# **VIII. RELATED PRODUCTS:**

Phospholipid Assay Kit (Colorimetric/Fluorometric) (K351)

Phosphatidylcholine Colorimetric/Fluorometric Assay Kit (K576)

Cardiolipin Assay Kit (Fluorometric)

Cholinesterase Activity Assay Kit (Colorimetric) (K975)

Phospholipase D (PLD) Activity Colorimetric Assay Kit (K725)

Choline/Acetylcholine Quantification Colorimetric/Fluorometric Kit (K615)

Pyruvate Kinase Activity Colorimetric/Fluorometric Assay Kit (K709)

Acetylcholinesterase Activity Colorimetric Assay Kit (K764)

Jurkat Cell Extract (1106)

HeLa Nuclear Extract (7947)

Choline Kinase B, Human Recombinant (P1220)

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