Choline Kinase (ChoK) Activity Assay Kit (Fluorometric)

(Catalog # BN00713; 100 assays, Store kit at -20°C)

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 roles 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 Assay Genie'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 µM of choline kinase activity.

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	BN00713	Cap Code	Part Number
Assay Buffer	50 ml	NM	BN00713-1
Substrate	1 vial	Green	BN00713-2
Detection Mix I	1 vial	Purple	BN00713-3
Detection Mix II	1 vial	Red	BN00713-4
ATP	1 vial	Orange	BN00713-5
Probe	0.4 ml	Blue	BN00713-6
ADP Standard (1 µmole)	1 vial	Yellow	BN00713-7
Human ChoK	10 µl	Brown	BN00713-8

V. User Supplied Reagents and Equipment:

- 96-well white flat-bottom fluorescent plate
- Multi-well spectrophotometer
- 10K Spin Column

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 before use. Store at 4°C.
- **Choline Substrate**: Reconstitute with 1.25 ml of dH_2O to generate choline solution. Keep on ice while in use. Store at -20°C.
- **Detection Mix I and Detection Mix II**: Reconstitute each vial with 220 µl 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_2O to generate ATP stock solution. Aliquot and store at -20°C. Keep on ice while in use.
- **Probe**: Thaw probe at room temperature before use. Store at -20°C. Use within two months.
- **ADP Standard**: Reconstitute with 1 ml of dH_2O to generate 1 mM ADP stock. Aliquot 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 10^6 -10^7) 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 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 enzyme 100-fold (eg. 2 µl in 198 µl of Assay Buffer). Add 5 µl of the diluted enzyme to a well. Adjust the volume to 50 µl 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) as background controls.

2. **ADP Standard:** Dilute the 1 mM ADP stock by 50 folds (eg. 20 µl in 980 µl of water) to prepare 10 µM ADP standard. Add 0, 2, 4, 6, 8, 10 µl of 20 µM ADP standard separately into the desired wells of a 96-well white flat-bottom plate to generate 0, 20, 40, 80, 120, 160, 200 pmole ADP/well. Adjust the volume to 50 µl 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	10 µl	-- µl
Detection I	2 µl	2 µl
Detection 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 and 50 µl Sample Background Mix to the samples designated as Sample Background Controls.

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 background control reading from its paired sample reading. Calculate the choline kinase activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$. Apply the ΔRFU to the ADP standard curve to get B pmole of ADP generated during the reaction time ($\Delta t = t_2 - t_1$).

$$\textbf{Specific Activity} = B \times D / (\Delta t \times V) = \text{pmol/min/ml (µU/ml)}$$

Where: **B** is ADP from 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.

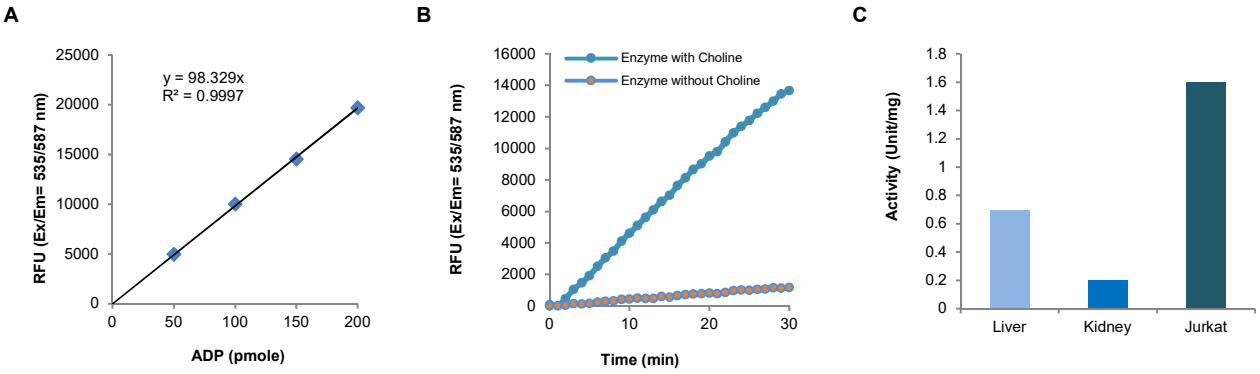

Figure A. ADP Standard Curve. **B.** Kinetic reaction rate of ChoK with and without choline substrate in the assay. **C.** Activity of ChoK determined by the assay in different samples.

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