Version 1b Last updated 1 December 2022

ab239729
Choline Kinase Assay Kit

For the measurement of choline kinase activity in different cell lines and tissues.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

In Choline Kinase Assay Kit (ab239729), 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.

2. Protocol Summary

Prepare sample, standards and background controls.

Prepare Reaction Mix and add 50 µL to the wells containing standards, samples and positive controls.

Add 50 µL Sample Background Mix to backgroung control wells.

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

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
Assay Buffer	50 mL	4°C
Substrate	1 vial	-20°C
Detection Mix I	1 vial	-20°C
Detection Mix II	1 vial	-20°C
ATP	1 vial	-20°C
Probe	0.4 mL	-20°C
Human ChoK	10 µL	-20°C
ADP Standard (µmole)	1 vial	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well plate with flat bottom.
- Multi-well spectrophotometer.
- 10K spin Column.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 Assay Buffer:

Ready to use as supplied. Bring it to room temperature before use. Store at 4°C.

6.2 Choline Substrate:

Reconstitute with 1.25 ml of dH_2O to generate choline solution. Keep on ice while in use. Store at -20°C.

6.3 Detection Mix I:

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.

6.4 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.

6.5 ATP:

Reconstitute with 220 µl of dH2O to generate ATP stock solution. Aliquot and store at -20°C. Keep on ice while in use.

6.6 Probe:

Thaw probe at room temperature before use. Store at -20°C. Use within two months.

6.7 ADP Standard:

Reconstitute with 1 ml of dH2O to generate 1 mM ADP stock. Aliquot and store at -20°C. Keep on ice while in use.

6.8 Human ChoK:

Ready to use as supplied. Store at -20°C. Use within two months.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Dilute the 1 mM ADP stock by 50 folds (eg. 20 µL in 980 µL of water) to prepare 20 µM ADP standard.

7.2 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, 40, 80, 120, 160, 200 pmole ADP/well.

7.3 Adjust the volume to 50 µL with Assay Buffer.

Standard #	20 µM ADP Standard (µL)	Assay Buffer (µL)	ADP/well
1	10	40	200 pmol
2	8	42	160 pmol
3	6	44	120 pmol
4	4	46	80 pmol
5	2	48	40 pmol
6	0	50	0 pmol

8. Sample Preparation

8.1 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.

8.2 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.

8.3 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.

8.4 Dilute it by 20-100 folds using the Assay Buffer. Add 5-10 µL of the diluted supernatant to a well.

8.5 **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.

Δ Note:

- For unknown samples, we recommend doing pilot experiments and testing several doses to ensure the readings are within the Standard Curve range.
- For samples exhibiting significant background, prepare sample well(s) as background controls.

9. Assay Procedure

9.1 Prepare 50 µL Reaction Mix for each well to be assayed as per the table and mix well. Add 50 µL of 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.

	Reaction Mix	Background Control Mix
Assay Buffer	33 µL	43 µL
Choline	10 µL	-
Detection I	2 µL	2 µL
Detection II	2 µL	2 µL
ATP	2 µL	2 µL
*Probe	1 µL	1 µL

Δ Note: *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.

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

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

10. Data Analysis

10.1 Subtract 0 ADP Standard slope from all Standard readings. Plot the ADP Standard Curve.

If sample background control slope is significant, then subtract sample background control reading from sample readings. 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 ($\Delta t = t_2 - t_1$).

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

Where: **B** is amount of ADP in the sample well from Standard Curve (pmol)

Δt is the Reaction time (min)

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

D is sample dilution factor

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

11. Typical Data

Typical data provided **for demonstration purposes only**.

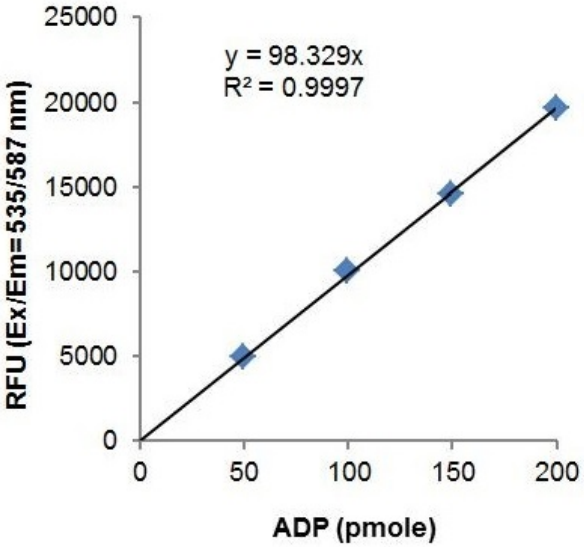

Figure 1. ADP Standard Curve.

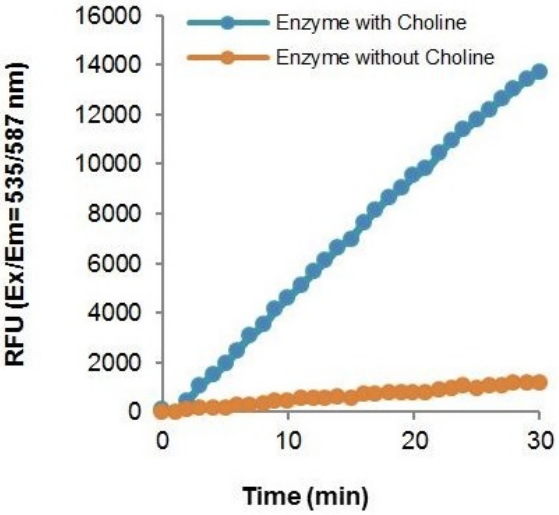

Figure 2. Kinetic reaction rate of ChoK with and without choline substrate in the assay.

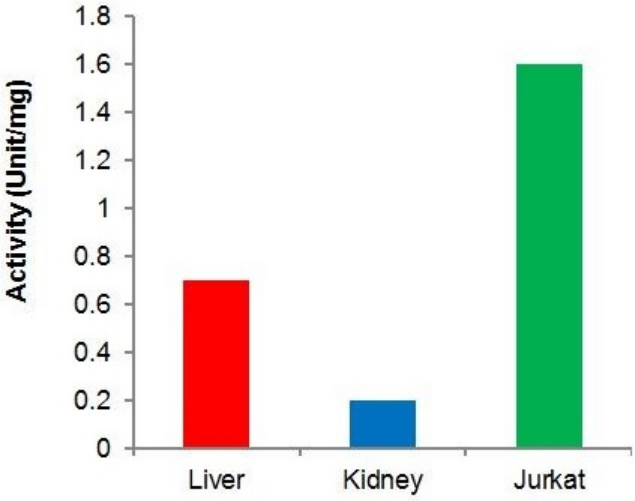

Figure 3. Activity of ChoK determined by the assay in different samples.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)