Recombinant Human Adenosine Kinase/ADK

Catalog Number: 8024-AK

DESCRIPTION

Source	*E. coli*-derived human ADK protein Ala2-His362 with a C-terminal 6-His tag Accession # P55263
N-terminal Sequence Analysis	Ala2
Predicted Molecular Mass	41 kDa

SPECIFICATIONS

SDS-PAGE	43-45 kDa, reducing conditions
Activity	Measured by its ability to phosphorylate Adenosine. The specific activity is >7 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: (10X) 250 mM HEPES, 1.5 M NaCl, 100 mM $MgCl_2$, 100 mM $CaCl_2$ pH 7.0 (supplied in kit)
- Recombinant Human Adenosine Kinase/ADK (Catalog # 8024-AK)
- Adenosine (Sigma, Catalog # A9251), 10 mM stock in deionized water
- Adenosine triphosphate (ATP) (Sigma, Catalog # A7699), 10 mM stock in deionized water
- Universal Kinase Activity Kit (Catalog # EA004)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare 1X Assay Buffer by diluting 10X Assay Buffer in deionized water.
2. Dilute 1 mM Phosphate Standard provided by the Universal Kinase Activity Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of 1X Assay Buffer for a 100 µM stock.
3. Prepare standard curve by performing seven one-half serial dilutions of the 100 µM Phosphate stock in 1X Assay Buffer. The standard curve has a range of 0.039 to 2.5 nmol per well.
4. Load 50 µL of each dilution of the standard curve into a plate in triplicate. Include a curve blank containing 50 µL of 1X Assay Buffer.
5. Prepare Substrate Mixture composed of 0.364 mM ATP and 3.64 mM Adenosine in 1X Assay Buffer.
6. Dilute rhADK to 50 ng/µL in 1X Assay Buffer.
7. Load 20 µL of the 50 ng/µL rhADK into the plate in triplicate. Include a control containing 20 µL of 1X Assay Buffer.
8. Add 55 µL of Substrate Mixture to the wells, excluding the standard curve and curve blank.
9. Incubate sealed at 37 °C for 1 hour.
10. Dilute Coupling Phosphatase 4 (supplied in kit) to 2.67 µg/mL in 1X Assay Buffer.
11. Add 100 µL of 1X Assay Buffer to each well containing the standard curve and curve blank.
12. Add 75 µL of 2.67 µg/mL Coupling Phosphatase 4 to wells containing enzyme and blanks, excluding the standard curve and curve blank.
13. Incubate sealed plate at room temperature for 5 minutes.
14. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
15. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
16. Read plate at 620 nm (absorbance) in endpoint mode.
17. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear fitting and adjusted for Substrate Blank.

**Use the sum of the incubation times prior to the addition of Malachite Green reagents due to the constant conditions throughout the assay.

Note: No coupling rate is applicable for this assay.

Final Assay Conditions

Per Reaction:

- rhADK: 1.0 µg
- Coupling Phosphatase 4: 0.2 µg
- ATP: 20 nmol
- Adenosine: 200 nmol

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

Adenosine kinase (ADK) converts adenosine into 5'-adenosine-monophosphate. ADK is therefore key in regulating the concentration of adenosine, an essential homeostatic and metabolic regulator in all living systems (1). Adenosine not only activates specific signaling pathways by binding to adenosine receptors but also is a primordial metabolite and regulator of numerous biochemical reactions that are related to metabolism and genetics. ADK dysfunction is involved in several pathologies, including diabetes, epilepsy, and cancer. For example, inhibiting ADK activity promotes the replication of primary insulin-producing β cells in diabetic mouse, rat, and pig (2); mutation of ADK causes hypermethioninemia, a condition with an excess of methionine in the blood (3); ADK is also identified as a neuropathological marker of the epileptic brain (4). Given its significant roles in various diseases, ADK is a rational therapeutic target (1). ADK is widely expressed in all tissues, with elevated levels in placenta, liver, muscle and kidney (5). The enzymatic activity of recombinant human ADK is measured using a phosphatase-coupled method (6).

References:

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3. Bjursell, M.K. *et al.* (2011) Am. J. Hum. Genet. **89**:507.
4. Aronica, E. *et al.* (2013) Neurochem. Int. in press.
5. Spychala, J. *et al.* (1996) Proc. Natl. Acad. Sci. U.S.A. **93**:1232.
6. Wu, Z.L. (2011) PLoS One **6**:e23172.

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