ADK Phosphorylation Assay Kit

Cat. No. Kit-0878 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	ADK Phosphorylation Assay Kit was specially designed to evaluate the phosphorylation of novel nucleoside analogues. The assay allows the test of 12 analogues at 7 different concentrations at the same time (or 6 analogues in duplicata). This assay is based on competitive inhibition of inosine (IR) phosphorylation by ADK enzyme. In the absence of nucleoside competitor, adenosine kinase phosphorylates inosine resulting in the formation of IMP. IMP is further oxidized by IMPDH to XMP and NADH2.This coupling reaction is immediate when IMPDH activity is much higher than ADK activity in the assay. The enzymatic activity of ADK, which corresponds to the formation kinetics of IMP, is then stoichiometrically and directly monitored by the formation kinetics of NADH2. In the presence of nucleoside competitor, the phosphorylation of inosine, poor ADK substrate, is inhibited detected as a decrease in NADH2 formation. This assay was validated with antiviral nucleoside analogues such as mizoribine, tubercidine, ribavirin.
Description	Human adenosine kinase enzyme is a purine salvage enzyme that has a broad substrate specificity and provides the phosphorylation of purine/pyrimidine nucleosides and pyrimidine ribonucleosides. Adenosine kinase is critical for phosphorylation of adenosine analogs and provides first steps of activation of highly effective anti-viral and immunosuppressive prodrugs, such as mizoribine, tubercidin and ribavirine.
Storage	ADK Phosphorylation Assay Kit must be stored at -20°C until used.

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Size	1 plate (96 assays)
Kit Components	A standard ADK Phosphorylation Assay Kit (one 96-well plate) contains: 1. one tube "IMPDH"; 2. one tube "Cofactor 1"; 3. one tube "Cofactor 2"; 4. one tube "Cofactor 3"; 5. one vial "Reaction Buffer 5x"; 6. one tube "Human ADK enzyme"; 7. Transparent 96-well plate.
Materials Required but Not Supplied	1) Plate agitator 2) Plate reader fitted with a filter 340nm.
Preparation	Important: Spin all tubes before solubilizin. 1. Preparation of Nucleoside analogues dilutions Prepare 7 different dilutions for each of the nucleoside analogue to be tested. Dispose 2µL of each dilution per well (use one column of the plate for one nucleoside analogue, the 8th well is used as a control with no analogue). 2. Reconstitute IMPDH enzyme Add 250µL of deionized water to "IMPDH" tube. Agitate gently until complete dissolution of the powder. 3. Preparation of standard reaction buffer (1x) i) Add the content of "Reaction Buffer 5x" tube to 16ml of deionized water to prepare "Reaction Buffer 1x". ii) Transfer quantitatively the content of 2 tubes with "Cofactor 1", "Cofactor 2" to the tube with "Reaction buffer 1x". Important: Do not add "Cofactor 3"! To do so: iii) -pipet 1ml of "Reaction buffer 1x" to each tube with cofactors and mix them by

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	inverting or pipeting up and down until the powder dissolved; iv) - transfer by pipeting the content of two tubes back into a vial "Reaction buffer 1x"; v) - repeat to be sure that all reagent and enzymes of the small tubes and vial are recovered. Mix by gently inverting until complete dissolution. Avoid bubbles. vi) Transfer quantitatively the content of "IMPDH" tube to "Reaction buffer 1x" with co-factors; vii) Solubilize the content of "Human ADK enzyme" tube by adding 1ml of complete "Reaction buffer 1x" with cofactors and IMPDH, transfer by pipeting the content of the tube back into a vial "Reaction buffer 1x". Composition of complete reaction buffer: 100mM Tris-HCl, 100mM KCl, 12mM MgCl2, BSA 1mg/ml, 5mM DTT, 5mM NAD, 0,1mM inosine, IMPDH 30mU/ml; human recombinant ADK 6mU/ml. 4. Preparation of ATP solution for starting the reaction Add 1ml of deionized water to the tube "Cofactor 3" containing ATP powder. Mix until dissolved. 50mM ATP solution is obtained.
Assay Protocol	1. Pre-incubation phase (15') i) Program the plate-reader in a kinetics mode with the measurements done every 1 minutes, absorbance at 340 nm, 37°C, agitation before the kinetics for 1 min, duration time 15min. ii) Add 200µL of standard reaction buffer per well. iii) Agitate and measure absorbance at 340nm (A340). Record this first set of data. 2. Start the reaction and incubate (30') i) Eject the plate from the plate-reader ii) Program the plate-reader in a kinetics mode with the measurements done every 1 minutes, absorbance at 340 nm, 37°C, agitation before the kinetics for 1 min, duration time 30-40min. iii) Start the reaction by adding 10µL of 50mM ATP per well (2.5mM ATP final concentration). iv) Place the plate in the plate-reader and start the measurements. Record second set

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