Continuous AMP Deaminase Assay Kit

Cat. No. Kit-0875 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	AMPD Assay Kit is the first non-radioactive and continuous kit designed to measure AMP deaminase content in samples. This enzymatic assay is based on a reaction involving Inosine Monophosphate Dehydrogenase (IMPDH). The principle of the assay is based on the coupling of the following enzymatic reactions: (1) In the presence of AMP, AMP Deaminase enzyme catalyzes the formation of IMP. (2) In the presence of NAD, IMP is immediately oxidized by a highly active IMPDH in the presence of NAD with simultaneous formation of NADH2 directly monitored spectrophotometrically at 340 nm. The assay is developed for measuring AMPDA activity in vitro or in cell lysates. For maximal accuracy, the assays with cell lysates are run with and without AMP in parallel. The absorbance rate observed in the absence of AMP is used as blank and is subtracted from the absorbance rate measured in its presence.
Description	Adenosine monophosphate deaminase is an enzyme that converts adenosine monophosphate (AMP) to inosine monophosphate (IMP), freeing an ammonia molecule in the process.
Storage	Once dissolved, the reagents provided in the kit are not stable and should be stored on ice and used the day of preparation.
Size	24 analysis
Kit Components	The kit allows to perform 24 analyses in a time (8 samples in triplicate, 12 samples in

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	duplicate). A standard AMP Deaminase Assay Kit contains: one tube "Cofactors" (DTT and NAD); one tube "IMPDH Enzyme"; one glass vail "Blank" (pre-filled with 10 ml of reaction buffer); one 15mL tube "Reaction mixture with AMP" (25μmol); one transparent 96-well plate.
Materials Required but Not Supplied	1) Plate agitator 2) Plate reader fitted with a filter 340nm, Epoch; PerkinElmer.
Preparation	Preparation of 10ml "Reaction mixture". 1. Reconstitute the enzymes with 200uL deionized water to the tubes with. Gently agitate until complete dissolution of the powder (avoid foaming). 2. Quantitatively transfer the content of 2 tubes with "Cofactors" and "IMPDH Enzyme" to "Blank" tube. To do so: - pipet 1ml of buffer from "Blank" to each tube and mix them by inverting or pipeting up and down until the powder is dissolved. - transfer the content of the tubes back into a vial "Blank" by pipeting. - repeat to be sure that all reagents and enzymes of the small tubes and vial are recovered. Mix by gently inverting until complete dissolution. Avoid bubbles. 3. Transfer 5ml of complete "Reaction mixture" containing enzymes and cofactors to orange cap 15ml tube pre-filled with AMP (powder). You have prepared: 5ml of "Blank"; 5ml of "Reaction mixture with AMP".
Assay Protocol	1. Preparation of hemolysates. The pellet of PBS-washed erythrocytes from 100μ of blood was frozenthawed twice, resuspended in 1mL of ice-cold deionized water and

	used directly for AMPDA quantification. 2. Add 4µ of hemolysates (indicated as S1-S11) per well as shown below: 3. Add 200µ of "Blank" per well and 200µ of "Reaction mixture with AMP" as shown below: 4. Program plate reader for kinetics absorbance reading (every 2min), 37°C. Insert the plate into the reader pre-heated at 37°C, agitate for 1min and monitor the reaction at 340nm at 37°C for 30min-1 hour with data collection every 2min.
Analysis	1. Calculate the absorbance rate per hour for reaction buffers with AMP (ARAMP) and without (ARblank). 2. Calculate MeanARAMP and MeanARblank. 3. Measure the concentration of hemoglobin [Hgb] in hemolysates using Drabkin's reagent and calculate final [Hgb] concentration used in assay. 4. AMPD activity is calculated by the following formula: Activity = (MeanARAMP-MeanARblank)/(4.9x[Hgb])x10^3 = (0.948-0.023)/(4.9x0.95)x10^3 = 199 nmol/hour/mg of Hgb where: MeanARAMP=0.948; MeanARblank=0.023; [Hgb], final haemoglobin concentration used in assay=0.95mg/ml. 4.9 is the absorbance of 1mM NADH at 340nm in 200µL-round-bottom well of 96-well microplate.

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