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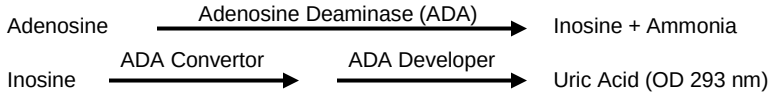
Adenosine Deaminase (ADA1) Inhibitor Screening Kit (Colorimetric)

11/16

Catalog # K993-100; 100 assays; Store kit at -20°C)

I. Introduction:

Adenosine Deaminase (ADA, E.C. 3.5.4.4.) is an enzyme that catalyzes the conversion of adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyinosine. Adenosine is a metabolite that plays important roles in determining cell proliferation, cell cycle progression, and immunosuppressive activation. Although multiple pathways exist for the production of adenosine, its deamination is irreversible, and becomes a critical point in purine metabolite signaling. Both adenosine and inosine bind the adenosine A_{2A} receptor yet can trigger different downstream signaling. Excess adenosine in penile tissue contributes to the disorder called priapism, and impaired adenosine signaling is associated with erectile dysfunction. For this reason, substantial work has gone into identification of inhibitors that would be of clinical relevance. The major isoform of adenosine deaminase, ADA1, is widely expressed in most cells in humans, particularly in lymphocytes and macrophages. ADA1 is present in the cytosol, nucleus and found associated with dipeptidyl peptidase-4 on the cell membrane while ADA2 was first found in the spleen but is predominantly found in the plasma and serum. Adenosine signaling is part of the immune response, and has been shown to reduce T-cell activation. Adenosine Deaminase activity is thus a marker for T-lymphocyte proliferation. BioVision's Adenosine Deaminase (ADA1) Inhibitor Screening Kit includes the larger 41 kDa form, human ADA1. In BioVision's ADA inhibitor screening kit, adenosine is deaminated by ADA1 forming inosine. This intermediate is converted to uric acid by a series of enzymatic reactions. The formed uric acid can be measured in the supplied U.V. transparent plate at OD 293 nm. A highly specific ADA1 inhibitor is included as a positive control for inhibition. The kit provides a rapid, simple, sensitive, and reliable test, suitable for high-throughput screening of ADA1 inhibitors.



II. Application:

- Screening/characterization ADA1 potential inhibitors

III. Kit Contents:

Components	K993-100	Cap Code	Part Number
ADA Assay Buffer (10X)	5 ml	WM	K993-100-1
ADA Convertor	1 vial	Green	K993-100-2
ADA Developer	1 vial	Blue	K993-100-3
ADA Substrate	500 µl	Brown	K993-100-4
ADA Enzyme	1 vial	Orange	K993-100-5
ADA Inhibitor (EHNA)	1 vial	Violet	K993-100-6
U.V. Transparent Plate (96-well)	1 plate	-	K993-100-7

IV. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer (capable of absorbance measurement)
- Anhydrous DMSO

V. Storage Conditions and Reagent Preparation:

Store kit at –20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read entire protocol before performing the experiment.

- **ADA Assay Buffer:** Make 1X buffer (i.e. mix 1 part 10X ADA Assay Buffer with 9 parts ddH_2O). Warm to RT before use. Store at -20 °C or 4 °C.
- **ADA Convertor and ADA Developer:** Reconstitute each with 210 µl **1X ADA Assay Buffer** and mix gently by pipetting. Store at -20°C. Avoid repeated freeze/thaw cycles.
- **ADA Substrate:** Ready to use. Warm ADA Substrate to RT. Aliquot and store at -20°C. Avoid repeated freeze/thaw.
- **ADA Enzyme:** Reconstitute with 550 µl of **1X ADA Assay Buffer** to prepare the stock solution. Store at -20°C. Avoid repeated freeze/thaw. Use within two months.
- **ADA Inhibitor:** Dissolve ADA Inhibitor (EHNA) in 100 µl ddH_2O to generate a 40X stock.

VI. ADA Inhibitor Screening Protocol:

1. **Screening compounds, Inhibitor Control (IC) and Enzyme Control (EC) preparations:** Dissolve test inhibitors into appropriate solvent; further dilute to 10X the desired test concentration with ADA Assay Buffer. For inhibitor control: dilute 5 µl of 40X ADA inhibitor into 15 µl **1X ADA Assay Buffer** to generate a 10X stock of ADA inhibitor. Add 10 µl 10X ADA inhibitor stock or 10X test compound to desired wells on the provided 96-well U.V. Transparent Plate.

   Note: Prepare parallel well(s) as Solvent Control to test the effect of the solvent on enzyme activity. In the instance that Solvent Control is significantly different from EC, use its values in the calculations below.

2. **ADA Enzyme Solution Preparation:** Prepare enough enzyme solution for the number of experiments to be performed. Per well, combine 40 µl of ADA Enzyme Solution:

   1X ADA Assay Buffer 35 µl

ADA Enzyme	5 µl

Mix Enzyme Solution well and then add to SC, Test Compound, and EC wells on supplied U.V. transparent plate. Incubate 30 minutes at 37°C before adding reaction mix to initiate reaction.

3. **Reaction Mix**: During incubation of enzyme with compounds and Solvent Controls, prepare enough reaction mix for the number of assays to be performed. Make 50 µl of reaction mix for each well containing:

	Reaction Mix
1X ADA Assay Buffer	44 µl
ADA Convertor	2 µl
ADA Developer	2 µl
ADA Substrate	2 µl

Add 50 µl of Reaction Mix to each well containing, Enzyme Control, Test Compound and Solvent Control. Mix well.

4. **Measurement:** Preincubate at 37°C for ten min. and then measure absorbance (OD 293 nm) in kinetic mode for at least thirty min. at 37°C. Choose two time points (t_1 and t_2) in the linear range (can be as short as 2 min.) of the plot and obtain corresponding absorbance for the sample (OD_{S1} and OD_{S2}).

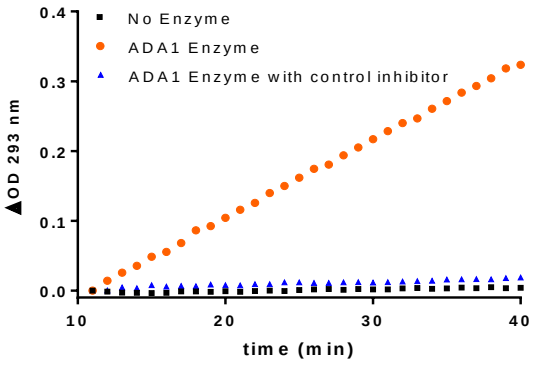
5. **Calculations:** Calculate the slope for all test inhibitor samples [S] by dividing the net ΔOD ($OD_{S2} - OD_{S1}$) values with the time interval Δt ($t_2 - t_1$). The EC is used to standardize the activity and should be run with each set of inhibitor screens[a]. Calculate % Relative Inhibition as follows:

$$\%\ \textbf{Relative Activity} = \frac{\textbf{Slope of Sample}}{\textbf{Slope of EC}} \times 100$$

$$\%\ \textbf{Relative Inhibition} = \frac{\textbf{Slope of EC} - \textbf{Slope of Sample}}{\textbf{Slope of EC}} \times 100$$

Notes:
If the solvent control shows substantially different kinetics from the EC, then the solvent control slope should be used in place of the EC slope for calculations.

a)



b)

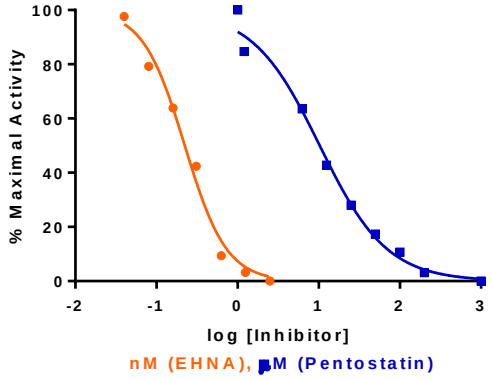


Figure: (a) Sample Inhibition of ADA1 enzyme activity by the supplied ADA Inhibitor. Reagent Background was subtracted for clarity. (b) Characteristic IC_{50} curves of relative inhibition as a function of inhibitor concentration. Curves generated using the ADA1 inhibitors Pentostatin (in blue) and erythro-9-(2-Hydroxy-3-nonyl)-adenine hydrochloride (EHNA) (orange). IC_{50} values obtained were 10.04 µM and 0.22 nM, respectively. Data were obtained by following the kit protocol.

VIII. RELATED PRODUCTS:

Adenosine Deaminase Activity Assay Kit (Colorimetric) (K321)
Adenosine Deaminase Activity Assay Kit (Fluorometric) (K328)
ADP Colorimetric Assay Kit II (K356)
Adenosine Antibody (6652)
3-Deazaadenosine (2771)
Xanthine Oxidase Colorimetric/Fluorometric Assay Kit (K710)
Purine Nucleoside Phosphorylase Activity Assay Kit (K767)
Adenosine Assay Kit (Fluorometric) (K327)
ATP Colorimetric/Fluorometric Assay Kit (K354)
ADP Colorimetric/Fluorometric Assay Kit (K355)
EHNA hydrochloride (2265)
Uric Acid Colorimetric/Fluorometric Assay Kit (K608)
Inosine Fluorometric Assay Kit (K712)
Ammonia Colorimetric Assay Kit II (K470)

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