ab284524 – Adenosine Deaminase Inhibitor Screening Kit (Colorimetric)

For the screening of Adenosine Deaminase inhibitors.
For research use only - not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284524

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
10X ADA Assay Buffer/ADA Assay Buffer	5 mL	-20°C
Converter Enzyme VI/ADA Convertor	1 vial	-20°C
Development Enzyme Mix VIII/ADA Developer	1 vial	-20°C
Human ADA1 Enzyme/ADA Enzyme	1 vial	-20°C
ADA Inhibitor/ADA Inhibitor (EHNA)	1 vial	-20°C
ADA Substrate	500 µl	-20°C
96-Well UV Transparent Plate/U.V. Transparent Plate (96-well)	1 unit	-20°C

Materials Required, Not Supplied

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

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

10X ADA Assay Buffer/ADA Assay Buffer: Make 1X buffer (i.e. mix 1-part 10X ADA Assay Buffer with 9 parts ddH_20). Warm to RT before use. Store at -20 °C or +4 °C.

Converter Enzyme VI/ADA Convertor and Development Enzyme Mix VIII/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.

Human ADA1 Enzyme/ADA Enzyme: Reconstitute with 550 µL 1X ADA Assay Buffer to prepare the stock solution. Store at -20 °C. Avoid repeated freeze/ thaw cycles. Use within two months.

ADA Inhibitor: Dissolve ADA inhibitor (EHNA) in 100 µL ddH_20 to generate 40X stock.

Assay Protocol

Screening Compounds, Inhibitor Control & Background Control preparations:

Test Samples [S]: Dissolve Test Sample(s) into appropriate solvent. Further dilute to 10X the desired test concentration with ADA Assay Buffer.

Inhibitor Control [IC]: 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.

Δ 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.*

Enzyme Control [EC] and Background Control [BC]: Add 10 µl of ADA Assay Buffer into designated well(s) of 96-Well UV Transparent Plate/96-well clear plate as Enzyme Control. Add 100 µl of ADA Assay Buffer into designated well(s) as Background Control. IC50 estimation (Optional): prepare several dilutions of selected candidate(s) in ADA Assay Buffer. Add 10 µl of each dilution into designated wells.

- **ADA Enzyme solution Preparation:** Mix enough enzyme solution for the number of assays to be performed. For each well, prepare a total of 40 µl Enzyme Mix containing:

	Enzyme Mix
1X ADA Assay Buffer	35 µl
Human ADA1 Enzyme/ADA Enzyme	5 µl

Mix Enzyme Solution well and then add to SC, Test Compound, and EC wells on supplied 96-Well UV Transparent Plate/U.V. transparent plate. Incubate 30 minutes at 37°C before adding reaction mix to initiate reaction.

- **Reaction Mix Preparation:** 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
Converter Enzyme VI/ADA Convertor	2 µl
Development Enzyme Mix VIII/ADA	2 µl

Developer	
ADA Substrate	2 µl

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

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 (t1 and t2) in the linear range (can be as short as 2 min.) of the plot and obtain corresponding absorbance for the sample (ODS1 and ODS2).

Calculation:

1. Calculate the slope for all test inhibitor samples [S] by dividing the net ΔOD (ODS2 – ODS1) values with the time interval Δt (t2 – t1). The EC is used to standardize the activity and should be run with each set of inhibitor screens.
2. Calculate % Relative Inhibition as follows:

$$\% \text{ Relative activity} = \frac{slope\ of\ (S)}{slope\ of\ (EC)} \times 100$$

$$\% \text{ Relative Inhibition} = \frac{slope\ of\ EC - slope\ of\ S}{slope\ of\ EC} \times 100$$

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)