ab204695 – Adenosine Deaminase (ADA) Activity Assay Kit (Fluorometric)

For the detection of ADA activity.
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/ab204695

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
10X ADA Assay Buffer/ADA Assay Buffer (10x)	25 mL	-20°C
ADA Convertor	1 vial	-20°C
Converter Enzyme VIII/ADA Developer	1 vial	-20°C
ADA Positive Control	1 vial	-20°C
OxiRed Probe/ADA Probe	200 µL	-20°C
ADA Substrate	500 µL	-20°C
Inosine Standard/Inosine Standard (10 mM)	100 µL	-20°C

Materials Required, Not Supplied

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

- 96-well plate with flat bottom. White plate is preferred for this assay.
- Fluorescence microplate reader
- Protease Inhibitor Cocktail
- Dounce homogenizer

Reagent Preparation

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

<u>10X ADA Assay Buffer/ADA Assay Buffer (10x):</u> Make 1x Assay Buffer by adding one part 10X ADA Assay Buffer/10x Assay Buffer to nine parts deionized water. Store at -20°C or 4°C. Bring to 37°C before use.

<u>ADA Convertor and Converter Enzyme VIII/ADA Developer:</u> Reconstitute each with 210 µl ADA Assay Buffer and mix gently by pipetting. Briefly centrifuge to collect the contents at the bottom of the tube. Aliquot and store at -20°C. Avoid repeated freeze/thaw.

<u>ADA Substrate:</u> Aliquot and store at -20°C. Avoid repeated freeze/thaw.

<u>ADA Positive Control:</u> Reconstitute with 22 µl ADA Assay Buffer and mix gently by pipetting. Briefly centrifuge to collect the contents at the bottom of the tube. Aliquot and store at -20°C. Avoid repeated freeze/thaw.

Assay Protocol

- Equilibrate all materials and prepared reagents to room temperature prior to use.

Sample preparation:

1. Rinse tissue and transfer ~100 mg of fresh or frozen tissue (stored at -80°C) to a pre-chilled homogenizer.
2. Add 300 µl cold ADA Assay Buffer containing protease inhibitor cocktail (not provided) and thoroughly homogenize tissue on ice.
3. Transfer the tissue homogenate to a cold microfuge tube.
4. To prepare cell extract, add 150-300 µl cold ADA Assay Buffer containing protease inhibitor cocktail (not provided) to 1-5 x 106 fresh or frozen cells and pipette several times to disrupt the cells.
5. Transfer cell homogenate including cell debris to a cold microfuge tube and agitate on a rotary shaker at 4°C for at least 15 min.
6. Centrifuge the tissue or cell homogenate at 16,000 X g, 4°C for 10 min.
7. Transfer the clarified supernatant to a fresh pre-chilled tube & store on ice. Use lysates immediately to assay ADA activity.

Δ Note: Lysates can be aliquoted and snap frozen in liquid nitrogen before storing at -80°C. Avoid freeze/thaw.

Inosine Standard:

1. Dilute Inosine Standard to 1 mM by adding 10 µl of Inosine Standard/10 mM Inosine Standard to 90 µl ADA Assay Buffer.
2. Further dilute the Inosine Standard to 10 µM by adding 10 µl of 1 mM Inosine to 990 µl ADA Assay Buffer.
3. Add 0, 2, 4, 6, 8 and 10 µl of diluted 10 µM Inosine Standard into a series of wells in a 96-well plate to generate 0, 20, 40, 60, 80 and 100 pmol/well Inosine Standard.
4. Adjust the volume to 50 µl/well with ADA Assay Buffer.

Adenosine Deaminase Activity Assay:

1. Add 2-50 µl of sample into desired well(s) in 96-well plate. For Positive Control, dilute the Positive Control 1:10 into ADA Assay Buffer and add 1-2 µl into desired well(s).
2. Adjust the volume of sample and Positive Control to 50 µl/well with ADA Assay Buffer.
3. Add 50 µl ADA Assay Buffer to one well as reagent Background Control.

Δ Notes:

a. For unknown samples, we suggest doing pilot experiment and testing several doses to ensure the readings are within the Standard Curve range.
b. Small molecules such as adenosine, inosine, xanthine, and hypoxanthine in the samples will contribute to the background. Remove these molecules by passing through a desalting column or by buffer exchange using a 10 kDa spin column. Use this modified sample for the assay. **Optional:** Prepare a parallel sample well as sample background control to ensure that these small molecules are removed by either using a desalting column or spin column.

Reaction Mix:

1. Prepare enough reagents for the number of assays to be performed. Make 50 µl of Reaction Mix and Background Control Mix containing:

Item	Reaction Mix	Background Control Mix
ADA Assay Buffer	40 µL	45 µL
ADA Convertor	2 µL	2 µL
Converter Enzyme VIII/ADA Developer	2 µL	2 µL
OxiRed Probe/ADA Probe	1 µL	1 µL
ADA Substrate	5 µL	-

2. Add 50 µl of Reaction Mix into each sample, reagent background control and Positive Control wells and 50 µl of Background Control mix to Standards and sample background control well(s). Mix well.

Measurement

Measure fluorescence (Ex/Em = 535/587 nm) in kinetic mode for at least 30 min. at 37°C. Choose two time points (T_1 & T_2) in linear range (can be as short as 2 min) of plot and obtain corresponding RFU for sample (RFU_{S1} and RFU_{S2}) and reagent background control (RFU_{BG1} and RFU_{BG2}). Read the Inosine Standard Curve along with the samples.

Calculations

1. Subtract 0 Standard reading from all Standard Readings. Plot the Inosine Standard Curve.
2. Subtract reagent background control reading from sample reading.
3. Apply the ΔRFU [(RFU_{S2}- $RFUB_{G2}$) – (RFU_{S1}- RFU_{BG1})] to the Standard Curve to get B pmol of Inosine generated by the sample during the reaction time ($\Delta T = T_2 - T_1$).

Δ Note: Sample background control reading should be less than reagent background control reading. We recommend removing the small molecules again using desalting column or a 10 kDa spin column if sample background control reading is higher than reagent background control**.**

$$Sample\ ADA\ Activity = \frac{B}{\Delta T \times \mu g\ of\ protein} \times \mathrm{DF} = \mathrm{pmol/min/\mu g}\ =\ \mathrm{\mu U/\mu g}$$

Where: **B** = Inosine amount from the Standard Curve (pmol)
ΔT = Reaction time (min)
µg of protein = Amount of protein/well (µg)
DF = Dilution factor of the sample

Technical Support

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