BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 955 Main St., Suite 4102, Buffalo, NY 14203
Tel: (716) 8293106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Adenosine Deaminase (ADA) Assay Kit (Cat #: E-164)

COMPONENTS:
ADA Reaction- 1 ml (for 100 samples), store at room temperature
ADA Control- 1 ml, store at store at room temperature
DA Reagent 1- 30 ml, store at 4ºC
DA Reagent 2- 6 ml, store at 4ºC
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The adenosine deaminase (ADA) activity assay kit is based on measurement of ammonia released from the ADA-mediated deamination reaction. The molar extinction coefficient for the colored product of the Berthelot reaction is ~20.8 $mM^{-1}cm^{-1}$ at 630-660 nm, allowing for measurement of crude cell/tissue ADA activity. Kit components are stable for at least one if stored and handled properly.

PROTOCOLS

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with dH_2O. Bring up ~10^5 washed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 mg tissue in 0.5 ml).

2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.

3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.5 - 4 mg/ml. *Normalization of sample protein concentration is recommended.*

Enzyme assay

1. Thaw protein samples and keep on ice (do not over thaw). Add 50 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate (for control and reaction).

2. After all samples have been added to plate, add 10 µl ADA Control to one set of wells and 10 µl ADA Reaction to the other set of wells. Gently agitate plate for 10 sec to mix contents. Cover plate and incubate in a 37°C incubator for 60 min. *Do not use CO_2 incubator.*

3. Remove plate from incubator after incubation. Prepare DA solution by mixing 5 parts of DA Reagent 1 and 1 part of DA Reagent 2, e.g., 1 ml DA Reagent 1 + 0.2 ml DA Reagent 2. Use the solution immediately. Discard unused solution.

4. Add 0.15 ml DA solution to each control well and reaction well. Gently agitate plate for 10 sec to mix contents. Cover plate and incubate in a 37°C incubator for another 30 min for color development.

5. Remove plate from incubator after incubation. Measure O.D. (absorbance) at 630-660 nm using a plate reader. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation.

6. ADA enzyme activity in IU/L = µmol/(L·min) = **ΔO.D.** × 1000 × 210 µl / (60 min × 0.7 cm × 20.8 × 50 µl) = **ΔO.D.** × **4.81**. Enzyme activity may be normalized by GAPDH enzyme activity (#E-101) or protein concentration (#A-117).

Additional information:

- The assay kit contains TX-100, salicylic acid, sodium nitroprusside, sodium hypochlorite, and NaOH. Please refer to the product page of our website or contact us for SDS information.
- 10x Cell Lysis Solution precipitates upon freezing. Redissolve contents by agitation in warm water upon arrival.