# ADA Activity Assay Kit

ARG83559 ADA Activity Assay Kit can be used to measure ADA in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, other biological fluids

Catalog number: ARG83559

Package: 100 tests

# TABLE OF CONTENTS



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## PRINCIPLE OF THE ASSAY

ARG83559 ADA Activity Assay Kit provides a simple and sensitive method for monitoring Adenosine Deaminase activity in various samples. In this assay, Adenosine Deaminase catalyzes conversion of substrate into inosine and an intermediate, which reacts with a developer to form a colored product that absorbs maximally at 620 nm.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store Positive Control at-20 °C, all other component at 2-8°C. Use the kit before expiration date.

| Component | Quantity | Storage |
|---|---|---|
| Microplate | 1 X 96-well plate | |
| Standard (1µmol/mL) | 1 ml | 4 °C |
| Positive Control | 1 vial (lyophilized) | -20 °C |
| Assay Buffer | 4 x 30 ml | 4 °C |
| Substrate | 1 vial (lyophilized) | 4 °C |
| Reaction Buffer | 10 ml | 4 °C |
| Dye Reagent A | 1 vial (lyophilized) | 4 °C |
| Dye Reagent B | 1 vial (lyophilized) | 4 °C |
| Dye Reagent B Diluent | 3 ml | 4 °C |
| Plate sealer | 3 adhesive strips | |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 620 nm
- Pipettes and pipette tips
- Deionized or distilled water

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Positive Control at-20 °C, all other component at 2-8°C. Use the kit before expiration date.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and Bacteria-Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation. Mix and sonicate with 1 ml Assay buffer per $5 \times 10^6$ cell or bacteria. Centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue- Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

## REAGENT PREPARATION

- **Substrate:** Reconstitute the **Substrate** with **1 ml** of Reaction Buffer. Allow the **Substrate** keep on bench for few minutes. Make sure the **Substrate** is dissolved completely and mixed thoroughly before use.
  The Reconstitute Substrate is stable for 4 weeks at-20°C.

- **Dye Reagent A:** Reconstitute the **Dye Reagent A** with **7 ml** of distilled water. Allow the **Dye Reagent A** keep on bench for few minutes. Make sure the **Dye Reagent A** is dissolved completely and mixed thoroughly before use.
  The Reconstitute Dye Reagent A is stable for 1 week at 4°C.

- **Dye Reagent B:** Reconstitute the **Dye Reagent B** with **3 ml** of Dye Reagent B Diluent. Allow the **Dye Reagent B** keep on bench for few minutes. Make sure the **Dye Reagent B** is dissolved completely and mixed thoroughly before use.
  The Reconstitute Dye Reagent B is stable for 1 week at 4°C.

- **Standard:** Perform 2-fold serial dilutions of the top standards to make the standard curve.

- **Positive Control:** Reconstitute the **Positive Control** with **1 ml** of Assay Buffer. Allow the **Positive Control** keep on bench for few minutes. Make sure the **Positive Control** is dissolved completely and mixed thoroughly before use.
  The Reconstitute **Positive Control** is stable for 1 week at -80°C.

## ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add 80 µl **Reaction Buffer** into each Sample, Control, Positive Control wells.
2. Add 10 µl **Sample**, **Distilled water**, **Positive Control** into respective wells.
3. Add 10 µl **Substrate** into Sample, Control and Positive Control wells.
4. Add 100 µl **Standard** into Standard wells.
5. Add 100 µl **Distilled water** into Blank wells.
6. Add 70 µl **Dye Reagent A** into each wells.
7. Add 30 µl **Dye Reagent B** into each wells.
8. Mix well Incubate for 10 min at RT. Read the OD at 620nm.

Summary of ADA Activity Assay Kit Procedure

| Reagent | Sample | Control | Standard | Blank | Positive Control |
|---|---|---|---|---|---|
| Reaction Buffer | 80 µl | 80 µl | - | - | 80 µl |
| Sample | 10 µl | - | - | - | - |
| Distilled water | - | 10 µl | - | - | - |
| Positive Control | - | - | - | - | 10 µl |
| Substrate | 10 µl | 10 µl | - | - | 10 µl |
| Standard | - | - | 100 µl | - | - |
| Distilled water | - | - | - | 100 µl | - |
| Dye Reagent A | 70 µl | 70 µl | 70 µl | 70 µl | 70 µl |
| Dye Reagent B | 30 µl | 30 µl | 30 µl | 30 µl | 30 µl |
| Mix well Incubate for 10 min at RT<br>Read the OD at 620 nm | | | | | |

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

a.) Definition: One unit of Adenosine Deaminase activity is defined as the enzyme deaminate 1 µmol of adenosine to inosine per min at pH 7.4 at 25° C.

$C_{Standard}$: the concentration of standard, 1 mmol/L = 1 µmol/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

$V_{Standard}$: the volume of the standard, 0.1 ml;

$V_{Sample}$: the volume of sample, 0.01 ml;

$V_{Assay}$: the volume of Assay buffer, 1 ml;

T: the reaction time, 10 minutes

b.) Calculation:

Formula:

a). According to the protein concentration

$$ADA\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample}) \times T]$$

$$= (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times C_{Protein}]$$

b). According to the weight

$ADA\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times W / V_{assay}) \times T]$

$= (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times W]$

c). According to the quantity of cell or bacteria

$ADA\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times N / V_{assay}) \times T]$

$= (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times N]$

4. According to the volume

$ADA\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times V_{assay} \times T]$

$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$