Aldo-Keto Reductase (AKRs) Assay Kit

Catalog No.: abx294427

Size: 96 tests

Detection Range: 0.05 U/L – 45 U/L

Sensitivity: 0.05 U/L

Storage: Store all components at -20°C in the dark.

Application: For detection and quantification of Aldo-Keto Reductase (AKRs) activity in serum, plasma, and tissue homogenates.

Introduction

Aldo-keto reductases (AKRs) are a family of enzymes found across both prokaryotic and eukaryotic organisms, with numerous isoforms identified. These enzymes catalyze NADPH-dependent reduction of carbonyl compounds to their corresponding alcohols, playing a critical role in detoxification and metabolic pathways. AKRs have also been implicated in various disease processes, including cancer.

Abbexa's Aldo-Keto Reductase (AKRs) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating AKRs activity. The substrate is catalyzed by AKRs, and the product reacts with a chromogenic reagent to produce a compound with an with an absorbance maximum at 450 nm. The intensity of the color is proportional to the AKRs activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 20 ml
3. Substrate: 0.2 ml
4. Standard: 1 vial
5. Plate sealer: 2

Materials required but not provided

1. Microplate reader (450 nm)
2. Double-distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly. If not tested immediately, samples can be stored at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh at least 20 mg of tissue and wash in cold PBS (0.01 m, pH 7.4). Add 180 µl of PBS (0.01 M, pH 7.4) per 20 mg of tissue and homogenize by hand, using a mechanical homogenizer at 4°C. Centrifuge the homogenate at 10,000 x g for 10 min at 4°C. Collect the supernatant, keep on ice and assay immediately. The protein concentration in the supernatant should be determined separately (**abx097193**)

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse lung tissue homogenate	1
Mouse plasma	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

Equilibrate all reagents to room temperature before use.

- **Working Solution:** Prepare sufficient Working Solution for the number of wells to be tested. Combine Substrate and Buffer Solution in a ratio of 1 : 160 and mix fully. Prepare immediately before carrying out the assay, keep in the dark, and use within 10 minutes of preparation.
- **500 µmol/L Standard:** Dissolve one vial of standard with 2 ml of double-distilled water and mix fully. Prepare immediately before carrying out the assay, keep in the dark, and use within 2 hours of preparation.
- **Standard Dilutions:** Label 8 tubes with 0 µmol/L, 100 µmol/L, 200 µmol/L, 250 µmol/L, 300 µmol/L, 350 µmol/L, 400 µmol/L, and 500 µmol/L. Prepare Standard Dilutions according to the following dilution scheme:

Standard Dilution (µmol/L)	**0**	**100**	**200**	**250**	**300**	**350**	**400**	**500**
500 µmol/L Standard (µl)	0	40	80	100	120	140	160	200
Double-distilled water (µl)	200	160	120	100	80	60	40	0

B. Assay Procedure

1. Mark microplate wells for each standard, sample, and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all wells in duplicate.*
2. Add 20 µl of standard dilutions to the standard wells.
3. Add 20 µl of sample to the sample wells.
4. Add 20 µl of sample to the control wells.
5. Add 180 µl of Chromogenic Working Solution to the standard and sample wells.
6. Add 180 of Buffer Solution to the control wells.
7. Incubate at 37°C for 30 minutes in the dark.
8. Measure the OD value of each well with a microplate reader at 450 nm.

C. Calculation of Results

Average the duplicate readings for each standard dilution. Subtract the mean OD value of the control well from each standard dilution to get the absolute OD values. Plot the standard curve, using the OD of the standard dilutions on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the activity of Aldo-Keto Reductase in each sample well can be derived with the following formulae:

1. Serum and Plasma samples:

One unit of Aldo-Keto Reductase activity is defined as the amount required for 1 L of serum or plasma to produce 1 µmol of NADPH per minute at 37°C.

$$\text{AKRs activity (U/L)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - \text{b})}{\text{a} \times \text{t}} \times \frac{\text{F}}{1000}$$

2. Tissue samples:

One unit of Aldo-Keto Reductase activity is defined as the amount required for 1 g of tissue to produce 1 µmol of NADPH per minute at 37°C.

$$\text{AKRs activity (U/g protein)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - \text{b})}{\text{a} \times \text{t} \times \text{C}_{\text{Protein}}} \times \frac{\text{F}}{1000}$$

where:

$\text{OD}_{\text{Sample}}$	OD value of sample
$\text{OD}_{\text{Control}}$	OD value of control
$\text{C}_{\text{Protein}}$	Concentration of protein in sample (g protein/L)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
t	Time of the enzymatic reaction (30 mins)
F	The dilution factor of sample
1000	1 mmol/L = 1000 µmol/L.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.