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ab273276 Aldose Reductase Activity Assay Kit (Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273276
(use https://www.abcam.cn/ab273276 for china, or
https://www.abcam.co.jp/ab273276 for Japan)

For the determination of Aldose Reductase activity in cell and tissue lysates and of purified Aldose Reductase enzyme.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Aldose Reductase Activity Assay Kit (Colorimetric) (ab273276) utilizes the ability of Aldose Reductase (AR) to catalyze the oxidation of NADPH. The reaction progress is followed by monitoring the decrease in absorbance at 340 nm. The assay has been optimized to be monitored using a 96 well plate. The kit can detect as low as 0.1 mU. The assay kit is simple and can be used in a high-throughput format.

2. Protocol Summary

Prepare lysates as directed and measure protein concentration

Prepare reagents as directed

Prepare standard curve

Add Positive Control, Samples and Background Control to appropriate wells

Add NADPH to Positive Control, Samples and Background Control wells

Add Reaction Mix (to Sample and Positive control wells) and Backgound Reaction Mix (to Sample Background wells)

Measure absorbance at 340 nm in kinetic mode for 40 to 60 minutes at 37 °C. Standard Curve may be read in end-point mode

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
AR Assay Buffer	35 mL	-20°C
DTT I	400 µL	-20°C
AR Substrate	1 mL	-20°C
Active Aldose Reductase	1 vial	-20°C
NADPH V	1 vial	-20°C
96-Well UV Transparent Plate	1 unit	Ambient

7. Materials Required, Not Supplied

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

- Plate reader capable of reading absorbance at 340nm
- Dounce tissue homogenizer

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **AR Assay Buffer:**

Store at -20 °C or 4 °C. Bring to room temperature before use.

9.2 **DTT I:**

Store at -20 °C or 4 °C. Protect from light.

9.3 **Active Aldose Reductase:**

Reconstitute with 50 µL AR Assay Buffer containing 10 µM DTT I*. Pipette up and down to mix well. Aliquot and Store at -20 °C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.

*a) Prepare a 100-fold dilution of 1 M DTT I to 10 mM DTT I (i.e. Dilute 2 µL of DTT I stock solution with 198 µL dH_2O), mix well.

b) Prepare AR Assay Buffer containing 10 µM DTT I (i.e. Dilute 2 µL of 10 mM DTT I with 1998 µL AR Assay Buffer), mix well.

9.4 **AR Substrate:**

Store at -20°C. Protect from light.

9.5 **NADPH V:**

Reconstitute with 440 µL dH_2O to generate a 20 mM NADPH V Stock Solution. Aliquot and store at –20 °C. Keep on ice while in use.

9.6 **96-Well UV Transparent Plate:**
Store at room temperature.

10. Sample Preparation

Tissue/cell lysate preparation:

10.1 Homogenize tissue (~50-100 mg) or pelleted cells (1-2 x 10^7) ~100-300 µL ice-cold AR Assay Buffer containing 10 µM DTT I and keep on ice for 10 min.

10.2 Centrifuge the homogenate at 12,000 x *g*, 4°C for 10 min. collect the supernatant.

10.3 Small molecules may interfere with the assay. We recommend sample ultrafiltration using 10K spin columns.

10.4 Centrifuge samples at 10,000 x *g*, 4 °C, 10 min, discard the filtrate and collect the ultraconcentrate.

10.5 Add fresh AR Assay Buffer containing 10 µM DTT I and bring back the volume of ultraconcentrate to its initial volume.

10.6 Repeat this step 3-5 times and bring back the ultraconcentrate volume to its initial volume.

11. Standard Curve

11.1 Prepare a 10 mM solution of NADPH V/NADPH by diluting 20 µL of 20 mM NADPH V (see Section 9.5) with 20 µL of AR Assay Buffer.

11.2 Add 0, 2, 4, 6, 8 and 10 µL of 10 mM (10 nmol/µL) NADPH V into a series of wells in a 96-Well UV Transparent Plate and adjust the final volume to 200 µL/well with AR Assay Buffer. This will generate 0, 20, 40, 60, 80 and 100 nmol/well of NADPH V Standard respectively.

Standard #	10 mM NADPH V (µL)	AR Assay Buffer (µL)	NADPH V (nmol/well)
1	10	190	100
2	8	192	80
3	6	194	60
4	4	196	40
5	2	198	20
6	0	200	0

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

Δ **Note:** Equilibrate AR Assay Buffer to 37 °C prior to the assay.

Samples and Sample Background Control:

12.1 Add 2-50 µL of Sample in duplicate into desired well(s) in a 96-Well UV Transparent Plate.

Δ **Note:** For Unknown Samples, we suggest testing several doses to ensure the readings are within the Standard Curve range and rates (progress curve kinetics) are within the linear range.

AR Positive Control:

12.2 For Positive Control, add 2-8 µL of reconstituted Active Aldose Reductase into desired well(s) in duplicate.

12.3 For all Sample(s), Sample Background Control and AR Positive Control, adjust the volume to 100 µL/well with AR Assay Buffer containing 10 µM DTT I.

NADPH probe preparation:

12.4 Prepare an 18-fold dilution of NADPH V stock solution (i.e. Dilute 10 µL of NADPH V stock solution with 170 µL AR Assay Buffer), mix well.

12.5 Add 60 µL of diluted NADPH V to each well containing the test Samples, Sample Background Control, AR Positive Control. Mix well.

AR Substrate Mix:

12.6 Prepare enough reagents for the number of assays to be performed. For each well, prepare 40 µL of the Substrate Mix:

Component	Reaction Mix	Background Mix
AR Assay Buffer	30 µL	40 µL
AR Substrate	10 µL	-

12.7 Add 40 µL of the Reaction Mix to well(s) containing Sample(s) and Positive Control.

12.8 Add 40 µl of Background Mix to well(s) containing Sample Background Control, mix well.

12.9 Measure absorbance at 340nm in kinetic mode for 40-60 minutes at 37 °C.

12.1 Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD_1 and OD_2).

Δ **Note:** Final assay volume is 200 µL

Δ **Note:** The NADPH Standard Curve can be read in endpoint mode

13. Calculations

13.1 Subtract 0 Standard reading from all readings.
13.2 Plot the NADPH Standard Curve.
13.3 Calculate the Aldose Reductase activity of the test sample by $\Delta OD = OD_1 - OD_2$ within the linear portion of the curve at time points t_1 and t_2.
13.4 Apply the ΔOD to the NADPH Standard Curve to get B nmol of NADPH generated during the reaction time ($\Delta t = t_2 - t_1$).
13.5 Do the same for the Sample Background Control.

$$Sample\ AR\ activity = \frac{B\ test\ sample\ -\ B\ sample\ background\ control}{(\Delta t\ x\ M)}\ x\ D$$

Sample AR activity = nmol/min/mg = mU/mg

B = NADPH amount from Standard Curve (nmol)
Δt = Reaction time (minutes)
M = Sample total protein added to the reaction well (mg)
D = Dilution factor

Unit definition:
One unit of Aldose reductase Activity is the amount of enzyme that oxidizes 1.0 µmol of NADPH per min, at pH 7.0 at 37 °C.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

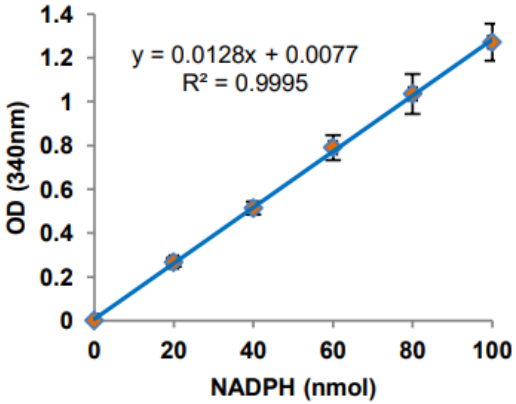

Figure 1. NADPH Standard Curve.

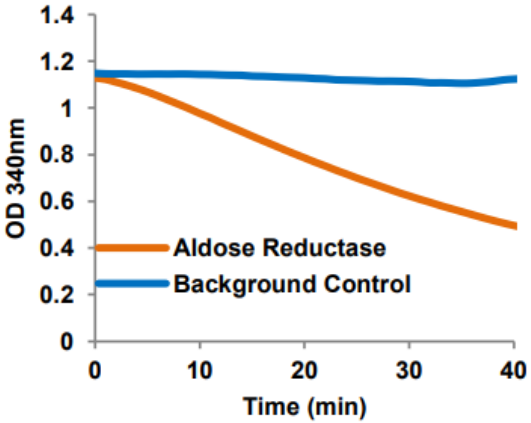

Figure 2. Purified Aldose Reductase activity.

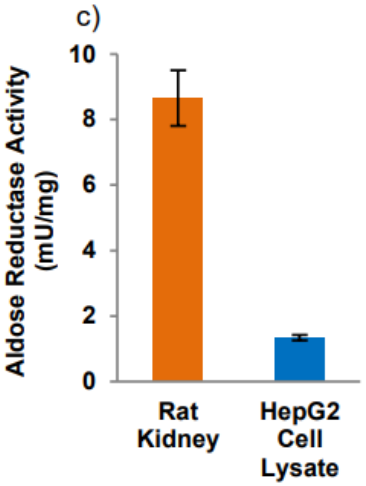

Figure 3. Aldose Reductase specific activity was calculated from Rat Kidney (20 µg protein) or HepG2 cell lysates (110 µg protein). Assays were performed following the kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

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)