Aldose Reductase Activity Kit (Colorimetric)

(Catalog # K369-100; 100 assays; Store at -20°C)

I. Introduction:

Aldose Reductase (Aldehyde Reductase; AR; ALR2; EC 1.1.1.21), a member of the aldo-keto reductase superfamily, catalyzes the NADPH-dependent reduction of a wide variety of aldoses (molecules containing carbonyl groups) to their corresponding alcohols. It is a key enzyme in the polyol (polyhydric alcohol) pathway and has been implicated to play a critical role in diabetes and cardiovascular complications. Aldose Reductase is the key factor in the reduction of glucose to sorbitol and its activity is higher during hyperglycemic conditions. The synthesis and accumulation of sorbitol due to AR activity is the main cause of diabetic complications, such as cataracts, retinopathy, neuropathy and nephropathy. Thus, AR could be used as a therapeutic target in the clinical treatment of secondary complications of diabetes. BioVision's Aldose Reductase Activity Kit is based on the ability of 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 assay is simple, sensitive and can detect as little as 0.1 mU of Aldose Reductase activity in a variety of sample types.

$$\text{Substrate} + \text{NADPH} + H^+ \xrightarrow{\text{Aldose Reductase}} \text{Product} + NADP^+ \ (\Delta\text{OD 340 nm Decrease})$$

II. Applications:

- Measurement of Aldose Reductase activity in various tissues/cells
- Analysis of polyol pathway

III. Sample Type:

- Tissue homogenates: Kidney, Spleen, etc.
- Cell culture: HepG2 cells, etc.
- Purified enzymes

IV. Kit Contents:

Components	K369-100	Cap Code	Part Number
AR Assay Buffer	35 ml	NM	K369-100-1
DTT (1 M)	0.4 ml	Green	K369-100-2
AR Substrate	1 ml	Red	K369-100-3
Aldose Reductase	1 vial	Purple	K369-100-4
NADPH	1 vial	Blue	K369-100-5
UV transparent plate (96-well)	1 plate	----	K369-100-6

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer (ELISA reader)
- Dounce Tissue Homogenizer (Cat. #1998 or equivalent)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protect from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. Upon opening, use within two months.

- **AR Assay Buffer:** Warm to room temperature before use. Store at 4 ºC or -20 ºC.
- **DTT & AR Substrate:** Store at -20 °C, protect from light.
- **Aldose Reductase:** Reconstitute with 50 µl AR Assay Buffer **containing 10 µM DTT***. 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.

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

  2) Prepare AR Assay Buffer containing 10 µM DTT (i.e. Dilute 2 µl of 10 mM DTT with 1998 µl AR Assay Buffer), mix well.
- **NADPH:** Reconstitute with 440 µl dH_2O to generate 20 mM NADPH Stock Solution. Aliquot and store at –20 °C. Keep on ice while in use.
- **U.V. transparent plate:** Upon receiving, store at room temperature.

VII. Aldose Reductase Assay Protocol:

1. Sample Preparation:

For Tissue and Cells: Rapidly homogenize tissue (~50-100 mg) or pelleted cells (~1 x 10^7) with ~100-300 µl ice-cold AR Assay Buffer **containing 10 µM DTT** and keep on ice for 10 min (*See Section VI, Aldose Reductase Notes*). Centrifuge samples at 12,000 x *g* at 4 ºC for 10 min and collect the supernatant. Small molecules may interfere with the assay. We recommend sample ultrafiltration using 10K spin column (BioVision: Cat. #1997). Centrifuge samples (10000 x *g*, 4 °C, 10 min), discard the filtrate and collect the ultraconcentrate. Add fresh AR Assay Buffer **containing 10 µM DTT** and bring back the volume of ultraconcentrate to its initial volume. Repeat this step 3-5 times and bring back the ultraconcentrate volume to its initial volume. **For Samples and Sample Background Control:** Prepare duplicate wells by adding 2-50 µl of prepared samples into well(s) of the provided 96-well UV transparent plate (labeled as "Sample" and "Sample Background Control").

For AR positive control: Add 2-8 µl of Reconstitute Aldose Reductase into desired well(s).

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**.

Notes: 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.

2. **NADPH Standard Curve:** Prepare 10 mM NADPH by diluting 20 µl of 20 mM NADPH Stock Solution with 20 µl AR Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl of 10 mM (10 nmol/µl) NADPH Standards into a series of wells in the provided 96-well UV transparent plate to generate 0, 20, 40, 60, 80, 100 nmol/well of NADPH Standard. Adjust the volume to **200 µl/well** with AR Assay Buffer.

   Note: Equilibrate AR Assay Buffer to 37 ºC prior to the assay.

3. **NADPH Probe Preparation:** Prepare an 18-fold dilution of NADPH stock solution (i.e. Dilute 10 µl of NADPH stock solution with 170 µl AR Assay Buffer), mix well. Add 60 µl of diluted NADPH to each well containing the test Samples, Sample Background Control, AR Positive Control. Mix well.

4. **AR Substrate Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total of 40 µl Reaction Mix containing:

	Reaction Mix	Background Mix
AR Assay Buffer	30 µl	40 µl
AR Substrate	10 µl	--

   Mix well and add 40 µl of the Reaction Mix to well(s) containing Sample(s) and Positive Control. Add 40 µl of Background Mix to well(s) containing Sample Background Control, mix well. ***Final Assay Volume: 200 µl***.

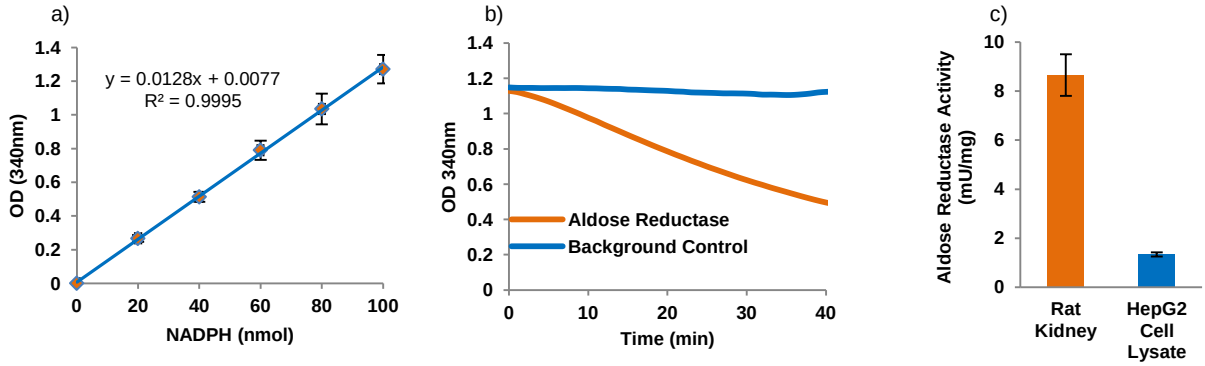
5. **Measurement:** Measure absorbance immediately at 340 nm in kinetic mode for 40-60 min at 37 ºC. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD_1 and OD_2). The NADPH Standard Curve (*See Step 2*) can be read in Endpoint mode.

6. **Calculation:** Subtract 0 Standard Reading from all Standard Readings. Plot the NADPH Standard Curve. Calculate the Aldose Reductase activity of the test sample: $\Delta OD = OD_1 - OD_2$ within the linear portion of the curve at time points t_1 and t_2. Apply the ΔOD to the NADPH Standard Curve to get B nmol of NADPH generated during the reaction time ($\Delta t = t_2 - t_1$). Do the same for the Sample Background Control.

$$\textbf{Sample Aldose Reductase Activity} = \frac{|(\textbf{B test sample} - \textbf{B sample background control})|}{\Delta t \cdot M} \times D = \textbf{nmol/min/mg} = \textbf{mU/mg}$$

Where: **B** = NADPH amount from Standard Curve (nmol)
Δt = reaction time (min.)
M = sample total protein amount added into 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 ºC.



Figures: (a) NADPH standard curve; (b) Purified Aldose Reductase activity; (c) 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.

VIII. RELATED PRODUCTS:

Aldose Reductase Inhibitor Screening Kit (K174)
Aldose Reductase, human recombinant (7361)
Sorbitol Dehydrogenase Activity Assay Kit (K935)
Dounce Tissue Homogenizer (1998)
D-Sorbitol Colorimetric Assay Kit (K631)
Epalrestat (2397)

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