TECHNICAL MANUAL

Aldose Reductase Inhibitor Screening Kit

- **SKU CODE:** ARMA00126
- **SIZE:** 100 wells
- **DETECTION PRINCIPLE:** Colorimetric (OD 340 nm)
- **RUO:** Research-Use-Only

Revised: 11/26

Aldose Reductase Inhibitor Screening Kit

Please read entire manual carefully before starting experiment.

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1. Key Features

Size:

100 wells

Detection method:

Colorimetric, OD 340 nm (kinetic)

Application:

Screening of potential aldose reductase inhibitors

Storage:

-20°C

2. Storage & Expiry

Store the unopened kit at -20°C. Bring all components to room temperature before use. Centrifuge all vials for a few seconds before opening.

DTT: Supplied as a 1 M solution.

Assay Buffer: Must be treated with DTT immediately before use. Dilute a portion of the DTT 1:1000 (1 µl < 1 ml DI water). Determine the volume needed for the number of assays to be performed (see procedure below) and add 10 µl for each 1 ml of buffer to be used. Store at 4°C.

D-Xylose: Ready to use as supplied. Store at -20°C.

Aldose Reductase: Reconstitute one vial with 110 µl Assay Buffer containing DTT, enough for 50 wells. Divide into convenient portions and store at -80°C.

NADPH: Reconstitute with 440 µl DI water. Divide into convenient portions and store at -20°C. Keep on ice while in use.

Epalrestat: Ready to use as supplied. Store at 4°C.

3. Product Description

Aldose Reductase catalyzes the NADPH-dependent reduction of an aldose (a sugar molecule containing a terminal aldehyde) to the corresponding alcohol. It is an important member of the polyol pathway, which is an alternative route for glucose metabolism that operates at high glucose levels. Accumulation of sorbitol due to AR activity, which is not sufficiently converted to fructose via sorbitol dehydrogenase, is believed to be the main cause of diabetic complications (cataracts, retinopathy, neuropathy, nephropathy). It causes osmotic stress in cells due to its inability to diffuse across cell membranes. Several aldose reductase inhibitors have shown promise in managing diabetic complications in clinical settings. Assay Genie's Aldose Reductase Inhibitor Screening Kit is used to evaluate potential inhibitors of aldose reductase.

Assay Principle:

Aldose reductase reduces xylose to xylitol while converting NADPH to NADP. NADP oxidation is followed at 340 nm as a decrease in absorbance. Inhibitors suppress the decrease in absorbance.

4. Applications

Evaluation of potential inhibitors of aldose reductase, including determination of IC50 values for test compounds.

5. Kit Contents

Component	Size	Cap Color
Assay Buffer	35 ml	NM
DTT	200 µl	Green
D-xylose	1 ml	Red
Aldose Reductase (2X)	Lyoph	Purple
NADPH	Lyoph	Blue
Epalrestat	20 µl	Yellow

6. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

7. Assay Procedure

Inhibitor Screening Protocol:

1. Turn on a plate reader and set to 37°C.
2. **Plate Preparation:** Determine the total number of wells to be run and prepare 200 µl of Assay Buffer for each well to be run. Include 1 or 2 Test Compound wells, a Background Control, Enzyme Control and Solvent Control for each compound to be tested. For each 200 µl of Assay Buffer, add 2 µl of the DTT solution. Prepare only the amount of buffer needed as DTT is unstable and will oxidize over time.

   Test Compounds (TC):

   a. Dissolve Test Compounds to 100X the final desired concentration in an appropriate solvent.

b. Transfer 10 µl of diluted Test Compound to a tube containing 40 µl Assay Buffer.

c. Transfer 10 µl of each Test Compound to wells of a 96-well plate designated as TC1, TC2, TC3, etc.

Inhibitor Control (IC):

a. Transfer 2 µl of Epalrestat to a tube containing 198 µl Assay Buffer and mix.

b. Transfer 10 µl of diluted Epalrestat to wells of the 96-well plate designated as Inhibitor Control.

Enzyme Control (EC):

Transfer 10 µl of Assay Buffer to wells of the 96-well plate designated as Enzyme Control.

Background Control (BC):

Transfer 100 µl of Assay Buffer to wells of the 96-well plate designated as Background Control.

Solvent Control (SC) (optional):

a. Transfer 10 µl of the solvent used for each test compound to a tube containing 40 µl Assay Buffer.

b. Transfer 10 µl of the diluted solvent to wells in the 96-well plate designated as Solvent Control.

The wells should have the following so far:

	TC	IC	EC	BC	SC
Test Compound(s)	10 µl	-	-	-	-
Diluted Epalrestat	-	10 µl	-	-	-
Assay Buffer	-	-	10 µl	100 µl	-
Diluted Solvent	-	-	-	-	10 µl

3. **NADPH Solution:** Each well requires 60 µl of NADPH solution, prepared by transferring 3.3 µl of NADPH Stock Solution to 57 µl Assay Buffer for each well to be run. Add 60 µl of the NADPH solution to all Test, Inhibitor Control, Enzyme Control, Background Control and Solvent Control wells.
4. **Aldose Reductase Solution:** Each well requires 90 µl of Aldose Reductase, prepared by transferring 2 µl of Aldose Reductase to 88 µl of Assay Buffer containing DTT for

each well to be run. Transfer 90 µl of the Aldose Reductase Solution to all Test, Inhibitor Control, Enzyme Control and Solvent Control wells. Shake the plate for several seconds to mix well and incubate at 37°C for 15 minutes to allow for inhibitor binding to take place.

5. **D-Xylose Solution:** Each well requires 40 µl of D-Xylose solution. Prepare sufficient material for the number of wells to be run, each well containing:

	D-Xylose Solution
Assay Buffer	36 µl
D-Xylose	4 µl

Mix well. Place at 37°C for 5-10 minutes to pre-equilibrate the temperature. Once the initial 15-minute incubation is complete, add 40 µl of the D-Xylose Solution to all wells. All wells should now contain 200 µl. Immediately place the plate in the plate reader previously set to 37°C.

6. **Measurement:** Begin acquiring absorbance data at 340 nm in kinetic mode for 30-60 minutes.

8. Calculation

For each well, identify a linear portion. There can be an initial lag phase lasting 2-5 minutes as the plate comes to 37°C, followed by a reasonably linear phase, then by a phase where the rate of decline slows as the NADPH concentration becomes limiting.

Calculate the slope of the identified linear phases for each well.

Subtract the slope of the Background Control from all Test Compound, Enzyme Control and Solvent Control slopes. If the Solvent Control is noticeably different from the Enzyme Control, use the Solvent Control slope as the control to be compared to the Test Compound slope rather than the Enzyme Control slope.

$$\% \text{ Inhibition} = 1 - [\ |\text{Slope}_{TC} - \text{Slope}_{BC}| \ / \ |\text{Slope}_{EC} - \text{Slope}_{BC}|\ ] \times 100$$

Or

$$\% \text{ Inhibition} = 1 - [\ |\text{Slope}_{TC} - \text{Slope}_{BC}| \ / \ |\text{Slope}_{SC} - \text{Slope}_{BC}|\ ]$$

if the Solvent Control slope is significantly different from the Enzyme Control slope.

It is nearly as accurate to simply take an absorbance value for all wells at a fixed time, such as 30 minutes, and use the absorbance rather than the slope.

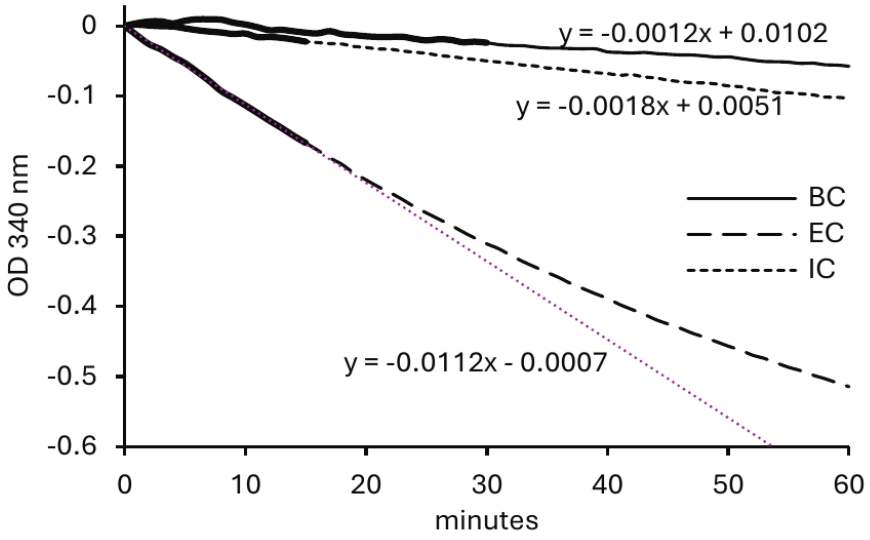
To determine IC50 values for the Test Compounds, perform a serial dilution on the Test Compound prior to transferring samples to the plate.

9. Materials Required (But Not Provided)

1. (Optional) UV-transparent 96-well plate.

10. Example

Example kinetic data at OD 340 nm for the Background Control (BC), Enzyme Control (EC) and Inhibitor Control (IC):



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Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.