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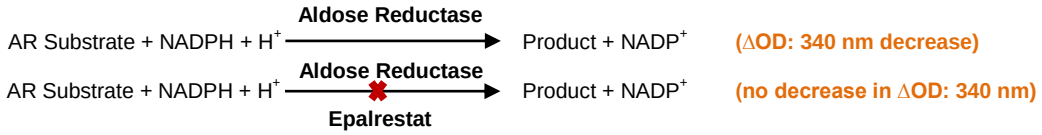
Aldose Reductase Inhibitor Screening Kit (Colorimetric)

03/19

(Catalog # K174-100; 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 pathway and the key factor in the reduction of glucose to sorbitol. Synthesis and accumulation of sorbitol due to AR activity is the main cause of diabetic complications, such as diabetic cataract, retinopathy, neuropathy and nephropathy. Inhibition of AR has been successfully used in clinical settings. These approved drugs prevent the accumulation of sorbitol. Thus, preventing eye and nerve damage in patients with diabetes. In addition, recent studies have shown that AR inhibitors may be able to prevent or delay the onset of cardiovascular complications such as ischemia/reperfusion injury, atherosclerosis and atherothrombosis. BioVision's Aldose Reductase Inhibitor Screening Kit can be used to screen potential inhibitors of this molecular target. Epalrestat, a noncompetitive AR inhibitor, is used as a Positive Control. In this assay, AR activity is monitored by the reduction in absorbance reading at OD 340 nm, while potential inhibitors arrest this decrease. The kit is adapted to a 96-well format and provides a rapid, simple, sensitive and reliable test for screening of AR inhibitors.



II. Applications:

- Screening/characterizing Aldose Reductase inhibitors

III. Kit Contents:

Components	K174-100	Cap Code	Part Number
AR Assay Buffer	35 ml	NM	K174-100-1
DTT (1 M)	0.4 ml	Green	K174-100-2
AR Substrate	1 ml	Red	K174-100-3
Aldose Reductase	1 vial	Purple	K174-100-4
NADPH	1 vial	Blue	K174-100-5
Epalrestat (10 mM/DMSO)	20 µl	Yellow	K174-100-6

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom, UV transparent plate is preferred
- Temperature-controlled plate reader

V. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protect from light. Briefly centrifuge small vials prior to opening.

- **AR Assay Buffer:** Warm to room temperature before use. Store at 4°C or -20°C.
- **DTT & AR Substrate:** Store at -20°C, protect from light.
- **Aldose Reductase:** Reconstitute with 100 µl AR Assay Buffer **containing 10 µM DTT*** to prepare Stock Solution. Aliquot Stock Solution and store at **-80 °C**. Once aliquoted, 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.
- **Epalrestat:** Ready to use. Bring to room temperature before use.

VI. Aldose Reductase Inhibitor Screening Protocol:

1. Screening Compounds, Inhibitor Control & Background Control preparations:

Test Sample(s) [S]: Dissolve Test Sample(s) to 100X in a proper solvent. Further dilute to 20X using AR Assay Buffer;

Inhibitor Control [IC]: Prepare a 100-fold dilution of Epalrestat (i.e. dilute 2 µl of Epalrestat with 198 µl AR Assay Buffer, mix well). Add 10 µl of diluted Test Sample(s), 10 µl of diluted Epalrestat into wells of 96-well clear plate designated as Test Samples [S] and Inhibitor Control [IC] respectively.

Enzyme Control [EC] and **Background Control [BC]:** Add 10 µl of AR Assay Buffer into designated well(s) of 96-well clear plate as Enzyme Control. Add 100 µl of AR Assay Buffer into designated well(s) as Background Control. **IC_{50} estimation (Optional):** prepare several dilutions of selected candidate(s) in AR Assay Buffer. Add 10 µl of each dilution into designated wells.

Note:

1) Various organic solvents may reduce the AR enzymatic activity. Prepare parallel well(s) as Solvent Control [SC] to test the effect of the solvent on AR activity in which the solvent concentration is the same as in your Test Sample(s). If [SC] slope is significantly different when compared to EC, use [SC] values to determine effect of the respective tested compound (see Step 6).

2) UV transparent plate is preferred.

	[S]	[IC]	[EC]	[BC]	[SC]
Test Sample	10 µl	-	-	-	-
Diluted Epalrestat	-	10 µl	-	-	-
AR Assay Buffer	-	-	10 µl	100 µl	-
Solvent Control	-	-	-	-	10 µl

2. **NADPH Probe Preparation:** Prepare an 18-fold dilution of NADPH Stock Solution (i.e. Dilute 20 µl of NADPH Stock Solution with 340 µl AR Assay Buffer), mix well. Add 60 µl of Diluted NADPH to each well containing Test Sample(s) [S], Inhibitor Control [IC], Enzyme Control [EC], Background Control [BC] and Solvent Control [SC].
3. **AR Enzyme Solution Preparation:** Prepare a 90-fold dilution of Aldose Reductase Stock Solution (i.e. Dilute 6 µl of Aldose Reductase with 534 µl of AR Assay Buffer containing 10 µM DTT). Mix thoroughly and keep on ice. Add 90 µl of Diluted Aldose Reductase Solution to each well containing Test Sample(s) [S], Inhibitor Control [IC], Enzyme Control [EC] and Solvent Control [SC]. *The total volume in every well is 160 µl/ per well.* Mix well and incubate at 37 °C for 15-20 min. Protect from light.

   Note: Always prepare fresh AR Assay Buffer containing 10 µM DTT *(See Section V, Aldose Reductase Notes).* Do not store Diluted Aldose Reductase Enzyme Solution.
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:

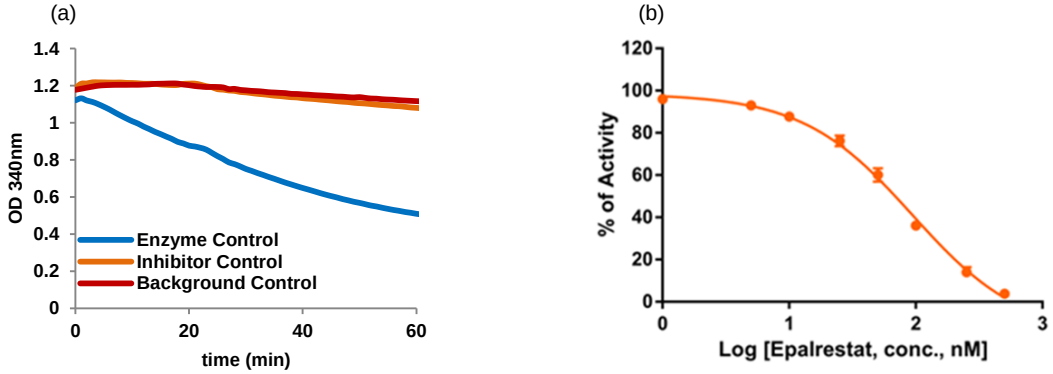
	Reaction Mix
AR Assay Buffer	36 µl
AR Substrate	4 µl

   Mix well. After the 15-20 min. incubation in *Step 3*, add 40 µl of the Reaction Mix to all well(s): Test Sample(s) [S], Inhibitor Control [IC], Enzyme Control [EC], Background Control [BC] & Solvent Control [SC] and mix well. ***Final Volume: 200 µl***.

   Note: Equilibrate AR Assay Buffer to 37 ºC prior to the assay.
5. **Measurement:** Measure absorbance immediately at 340 nm in kinetic mode for 60-90 min at 37 °C. 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).
6. **Calculation:** Calculate the slope for all Test Samples [S], Enzyme Control [EC], Solvent Control [SC] and Background Control [BC] by dividing the net ΔOD (A_1-A_2) values with the time Δt (t_2-t_1). Subtract the Slope of Background Control from [S], [EC] and [SC]. If [SC] slope is significantly different when compared to [EC], use [SC] values to determine effect of test compound.

$$\% \text{ Relative Inhibition} = \frac{|\text{Slope of [EC]} - \text{Slope of [S]}|}{|\text{Slope of [EC]}|} \text{X100}$$

$$\% \text{ Relative Activity} = \frac{|\text{Slope of [S]}|}{|\text{Slope of [EC]}|} \text{X100}$$



Figures: (a) Progress Curves of Aldose Reductase Enzyme Control and Inhibitor Control (Epalrestat). (b) Inhibition of Aldose Reductase activity by Epalrestat. IC_{50} of Epalrestat: 93.5 ± 1.9 nM. Assays were carried out following the kit protocol.

VII. RELATED PRODUCTS:

Aldose Reductase, human recombinant (7361)
Epalrestat (2397)
Sorbitol Dehydrogenase Activity Assay Kit (K935)
Aldose Reductase Activity Assay Kit (K369)
D-sorbitol Colorimetric Assay Kit (K631)
NAD/NADH Quantification Kit (K337)

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