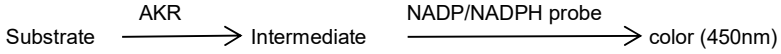
Aldo-keto Reductase Activity Assay Kit (Colorimetric) (#BN01063)

(Catalog #BN01063; 100 assays; Store at -20°C)

I. Introduction:

Aldo-keto Reductases (AKRs) are a superfamily of NAD(P)H linked oxidoreductases which contains more than 190 members. A complete listing of AKR superfamily can be found at: https://www.med.upenn.edu/akr/. The common function of AKR superfamily is to oxidize/reduce aldehydes or ketones to their corresponding primary or secondary alcohols. Due to their broad substrate specificity, AKRs play roles in intermediary metabolism, biosynthesis and detoxification. In humans, the AKRs are not only involved in carbonyl metabolism, but they also regulate signaling through nuclear receptors which leads to chemical carcinogenesis. Many studies demonstrate increasing expression of AKRs in cancers such as lung, liver and colon cancers. Assay Genie's Aldo-keto Reductase Activity Assay Kit provides a convenient tool for sensitive detection of AKR activity in a variety of samples. In this assay, AKR acts on a general substrate and converts $NADP^+$ to NADPH. This NADPH reacts with the AKR probe and generates color. This color is proportional to the activity of AKR in the sample. This kit can detect AKR activity as low as 0.001 mU in samples and has been validated with AKR1B10, AKR1C1 and AKR1C3. Further tests are in progress for other members of the AKR family.



II. Applications:

- Measurement of aldo-keto reductase activity in various tissues/cells.
- Screening of aldo-keto reductase inhibitors.

III. Sample Type:

- Animal tissues: such as liver, pancreas, small intestine, etc.
- Cells: Adherent and suspension cells.
- Biological fluids: such as serum and plasma.

IV. Kit Contents:

Components	BN01063	Cap Code	Part Number
AKR Assay Buffer	25 ml	WM	BN01063-1
AKR Substrate	10 ml	NM	BN01063-2
AKR Probe	1 vial	Red	BN01063-3
AKR Positive Control	10 µl	Violet	BN01063-4
NADPH Standard	1 vial	Yellow	BN01063-5

V. User Supplied Reagents and Equipment:

- 96-well clear flat bottom plate.
- Multi-well spectrophotometer.

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C, protected from light. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

- **AKR Assay Buffer and AKR Substrate:** Warm to room temperature before use. Store at either 4ºC or -20ºC.
- **AKR Probe:** Reconstitute with 0.9 ml of ddH2O. Pipette up and down several times to completely dissolve the pellet (Do not vortex). Aliquot and store at –20°C. Keep on ice while in use. Use within two months.
- **AKR Positive Control:** Aliquot and store at –20°C. Keep on ice while in use. Use within two months.
- **NADPH Standard:** Reconstitute NADPH Standard with 200 µl DMSO to generate 1 mM NADPH Standard solution. Aliquot and store at –20°C. Keep on ice while in use. Use within two months.

VII. Aldo-keto Reductase Activity Assay Protocol:

1. **Sample Preparation:** Homogenize ~10-50 mg tissue or ~1 x 10^6 cells in 200 µl ice-cold Assay Buffer. Centrifuge at 13,000 x g, 4°C for 10 min and collect the supernatant. Add 2 - 50 µl supernatant per well. For the AKR Positive Control, add 2-5 µl of positive control to desired well(s). For serum sample, 5 – 50 µl serum can be tested directly. Adjust the final volume of all samples to 100 µl with the Assay Buffer.
   Notes:
   a. For unknown samples, we suggest testing several doses to ensure the readings are within the linear range of the Standard Curve.
   b. For samples with significant background, prepare parallel sample well(s) as background controls (BCs).
2. **NADPH Standard Curve:** Dilute 1 mM NADPH Standard 1:5 to 0.2 mM NADPH by adding 20 µl NADPH Standard to 80 µl Assay Buffer. Add 0, 10, 20, 30, 40 µl of the 0.2 mM NADPH Standard into a series of wells in a 96-well plate to generate 0, 2, 4, 6, 8 nmol/well NADPH standards. Adjust the final volume to 100 µl with Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 100 µl Mix containing:

	Reaction Mix	*Background Control Mix
AKR Assay Buffer	0 µl	92 µl
AKR Substrate	92 µl	0 µl
AKR Probe	8 µl	8 µl

Mix and add 100 µl of the Reaction Mix to each well containing the Standard, Positive Control, and test samples.

* For background correction, add 100 µl of Background Control Mix (without substrate) to sample background control well(s) and mix well.

4. **Measurement:** Start measuring absorbance immediately at 450 nm in a kinetic mode for 10-120 min. at 37°C.

   Note:

   a) The NADPH Standard Curve can be read in Endpoint mode (after 10 min of incubation).

   b) Sample incubation time depends on the AKR activity in them. We recommend measuring the OD in a kinetic mode, and choosing two time points (T_1 & T_2) in the linear range of the standard curve to calculate the AKR activity of the samples.

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the NADPH Standard Curve. Calculate the AKR activity of the test samples by subtracting the absorbance reading at T2 and T1: $\Delta OD = A_2 - A_1$. If necessary subtract the $\Delta BC = BC_2 - BC_1$ from this reading. Apply the ΔOD to the NADPH Standard Curve to get B nmol of NADPH generated during the reaction time ($\Delta T = T_2 - T_1$).

Sample Aldo-keto Reductase Activity = B/ (ΔT X V) x D = nmol/min/ml = mU/ml

Where: **B** = NADPH amount from Standard Curve (nmol).
ΔT = reaction time (min.).
V = sample volume initially added into the reaction well (ml).
D = Dilution Factor

Unit Definition: One unit of aldo-keto reductase is the amount of enzyme that generates 1.0 µmol of NADPH per min. at pH 8.0 at 37°C.

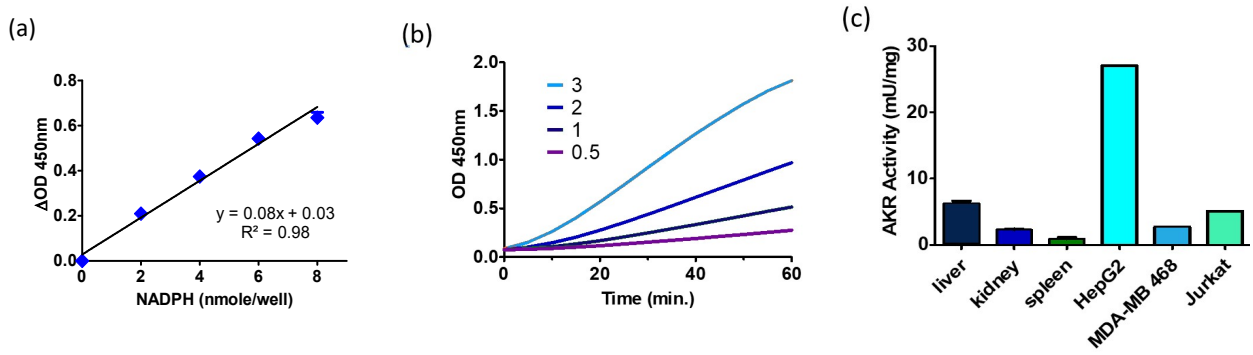


Figure: (a) NADPH standard curve; (b) AKR Positive Controls (µl/assay). (c) Human tissue samples and cancer cell line (HepG2: hepatocellular carcinoma; MDA-MB: adenocarcinoma; Jurkat: acute T cell leukemia) lysates were homogenized as described in the kit protocol. Serial dilutions were tested to ensure the readings were within the linear range of the Standard Curve. AKR activities (mU/mg) were measured and calculated as in the kit protocol. Assay Genie's AKR activity assay kit demonstrated enhanced AKR activities in the liver and liver cancer cell line lysates.

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