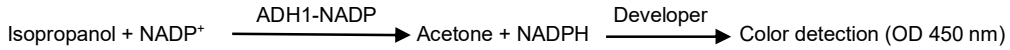
Isopropanol Dehydrogenase Activity Assay Kit (Colorimetric) (#BN00922)

(Catalog # BN00922; 100 assays; Store at -20°C)

I. Introduction:

NADP-dependent Isopropanol Dehydrogenase (ADH1-NADP, EC:1.1.1.80) belongs to the superfamily of alcohol dehydrogenases (Alcohol DHs, ADHs, EC 1.1.1.1) with a preference for medium chain secondary alcohols, such as 2-butanol and isopropanol. Isopropanol dehydrogenase facilitates the conversion between secondary alcohols to aldehydes and ketones with the reduction of NADP+ to NADPH. Assay Genie's Isopropanol Dehydrogenase Activity Colorimetric Assay Kit provides a convenient tool for sensitive detection of the ADH1-NADP in a variety of samples. In this assay, Isopropanol gets converted to Acetone and NADPH in the presence of $NADP^+$. This results in the development of colour in the presence of the developer. This colour is proportional to the ADH1-NADP levels in the sample and can be measured at λ = 450 nm. This assay detects ADH1-NADP activity as low as 0.001 mU in samples.



II. Applications:

- Measurement of Isopropanol Dehydrogenase (ADH1-NADP) activity in various cells.
- Measurement of NADP-dependent alcohol dehydrogenases' activity with broad substrate specificity in various tissues/cells.

III. Sample Type:

- Bacterial, yeast or protozoan samples: *Entamoeba histolytica*, *Clostridium beijerinckii*, *Mycoplasma pneumonia*, etc.
- Animal tissues: such as liver, pancreas, small intestine, etc.
- Animal cells.

IV. Kit Contents:

Components	BN00922	Cap Code	Part Number
ADH1-NADP Assay Buffer	25 ml	WM	BN00922-1
ADH1-NADP Substrate	1 ml	Blue	BN00922-2
ADH1-NADP Developer	1 vial	Red	BN00922-3
ADH1-NADP Positive Control	100 µl	Violet	BN00922-4
NADPH Standard	1 vial	Yellow	BN00922-5

V. User Supplied Reagents and Equipment:

- 96-well clear flat bottom plate.
- Multi-well spectrophotometer (plate/ELISA reader).

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.

- **ADH1-NADP Assay Buffer:** Warm to room temperature before use. Store at either 4ºC or -20ºC.
- **ADH1-NADP Developer:** Reconstitute the Developer with 0.9 ml of ddH_2O. 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.
- **ADH1-NADP Positive Control:** Dilute the Positive Control with 100 µl Assay Buffer and mix thoroughly. Aliquot and store directly at –20°C. Keep on ice while in use. Use within two months.
- **NADPH Standard:** Reconstitute NADPH Standard with 200 µl pure 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. Isopropanol Dehydrogenase Assay Protocol:

1. **Sample Preparation:** Homogenize ~10-50 mg tissues 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 and adjust the volume to 50 µl with the Assay Buffer. For the ADH1-NADP Positive Control, dilute the ADH1-NADP Positive Control 1:9 by adding 2 µl of Positive Control to 18 µl Assay Buffer. Add 2-10 µl of diluted positive control solution to desired well(s). For serum sample, 5 – 50 µl serum can be tested directly. Adjust the final volume of all samples to 50 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 10, 20, 30, 40 µl of the 0.2 mM NADPH Standard into a series of wells in a 96-well plate to generate 2, 4, 6, 8 nmole/well NADPH standards. Adjust the final volume to 50 µl with ADH1-NADP 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
ADH1-NADP Assay Buffer	82 µl	92 µl
ADH1-NADP Developer	8 µl	8 µl
ADH1-NADP Substrate	10 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: Incubation time depends on the isopropanol dehydrogenase activity in samples. 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 isopropanol dehydrogenase activity of the samples. The NADPH Standard Curve can be read in Endpoint mode (i.e., at the end of the incubation time).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the NADPH Standard Curve. Calculate the isopropanol dehydrogenase activity of the test sample by subtracting the absorbance reading at T2 and T1: $\Delta OD = A_2 - A_1$ and if necessary subtracting the $\Delta BC = BC_2 - BC_1$. 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 Isopropanol Dehydrogenase Activity = B/ (ΔT X V) x D = nmol/min/ml = mU/ml

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

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

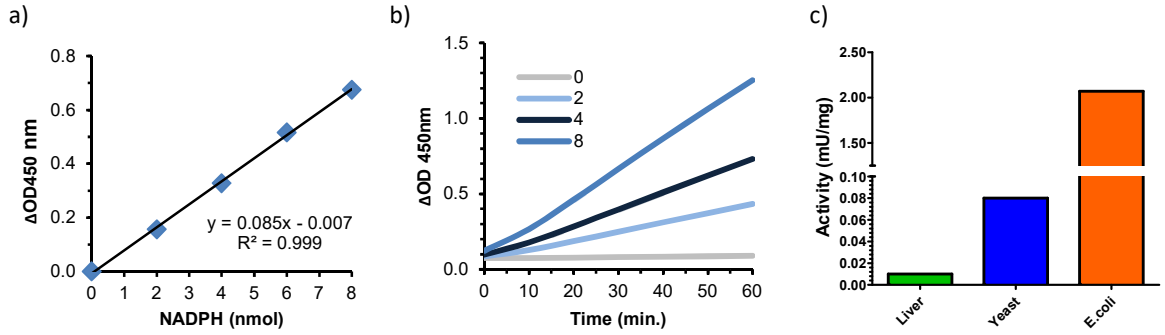


Figure: (a) NADPH standard curve; (b) ADH1-NADP Positive Controls (µl/assay). Assays were performed following the kit protocol; (c) ~5 mg of bovine liver, yeast and *E.coli* cell pellet were homogenized as described in the kit protocol. Serial dilutions were tested to ensure the readings were within the linear range of the Standard Curve. ADH1-NADP activities (mU/mg) of samples were measured and calculated as in the kit protocol.

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