Isocitrate Dehydrogenase Activity Colorimetric Assay Kit

(Catalog # BN00969; 100 reactions; Store kit at –20°C)

I. Introduction:

Isocitrate dehydrogenase (IDH; EC 1.1.1.41, NAD^+) is an enzyme that participates in the citric acid cycle. These IDH3 isoforms catalyze the oxidative decarboxylation of isocitrate, producing α-ketoglutarate and CO_2 while converting NAD^+ to NADH. This is a two-step process, which involves oxidation of isocitrate to oxalosuccinate, followed by the decarboxylation of the beta-carboxyl group to form the ketone, α-ketoglutarate. Other isoforms (EC 1.1.1.42, $NADP^+$) catalyze the same reaction, but unrelated to the citric acid cycle. It is carried out in the mitochondrion (IDH2) as well as in the cytosol and peroxisome (IDH1) and use $NADP^+$ as a cofactor instead of NAD^+. Assay Genie's Isocitrate Dehydrogenase Assay Kit provides a convenient tool for sensitive detection of NAD^+-dependent, $NADP^+$-dependent or both IDHs in a variety of samples. The IDHs utilize isocitrate as a specific substrate leading to a proportional color development and can be easily quantified colorimetrically (λ = 450 nm) with detection sensitivity as low as 0.01 mU.

II. Kit Contents:

Components	BN00969	Cap Code	Part Number
IDH Assay Buffer	25 ml	WM	BN00969-1
NAD^+ (lyophilized)	1 vial	Blue	BN00969-2
$NADP^+$ (lyophilized)	1 vial	Brown	BN00969-3
IDH Substrate (lyophilized)	1 vial	Red	BN00969-4
Developer (lyophilized)	1 vial	Purple	BN00969-5
IDH Positive Control ($NADP^+$)	20 µl	Green	BN00969-6
NADH Standard (0.5 µl mol, lyophilized)	1 vial	Yellow	BN00969-7

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow IDH Assay Buffer to warm to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

- Reconstitute the NAD^+, $NADP^+$, and IDH Substrate with 220 µlddH_2O separately.
- Reconstitute the Developer with 0.9 ml of ddH_2O. Pipette up and down several times to completely dissolve the pellet into solution (**Don't vortex**).
- Reconstitute the NADH Standard with 50 µl ddH_2O to generate a 10 mM NADH stock solution.
- All components are stable for up to 2 months at -20°C after reconstitution or freeze-thaw cycles (< 5 times).

V. IDH Assay Protocol:

1. **NADH Standard Curve:** Dilute 10 µl of the 10 mM NADH stock solution with 90 µl of Assay Buffer to generate a 1 mM NADH standard. Add 0, 2, 4, 6, 8, 10 µl of the 1 mM NADH standard into a 96-well plate in duplicate to generate 0, 2, 4, 6, 8, 10 nmol/well standards. Adjust the final volume to 50 µl with Assay Buffer. The NADH standard curve can be used as the standard of NAD^+ IDH as well as $NADP^+$ IDH.
2. **Sample Preparations:** Tissues (50 mg) or cells (1×10^6) can be homogenized in ~ 200 µl ice-cold Assay Buffer, then centrifuged (13,000 x g, 10 min) to remove insoluble material. 5 - 50 µl serum samples can be directly added into 96-well plate. Adjust the total volume of test samples to 50 µl/well with Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the linear range of the standard curve. For positive control (optional), add 2-5 µl positive control solution to wells and adjust to 50 µl with Assay Buffer. NAD(P)H in samples will generate background, so if NAD(P)H is in your sample, set up the background control group to avoid the interference (see next step).
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a Reaction Mix (50 µl) containing:

Reaction Mix	Background Control Mix
40 µl IDH Assay Buffer	42 µl IDH Assay Buffer
8 µl Developer	8 µl Developer
2 µl IDH Substrate	
2 µl NAD^+ or $NADP^+$ or both (then use 38 µl Assay Buffer)*	

Add 50 µl of the Reaction Mix to each well containing the test samples, positive controls, and standards or 50 µl of the Background Control Mix to background control well. Mix well.

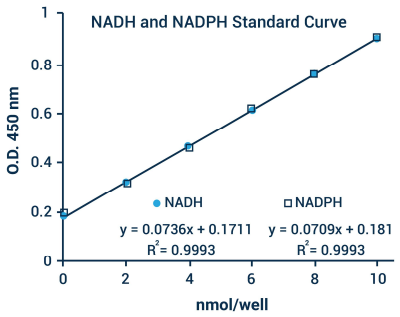
***Note:** Add NAD^+, the assay will detect NAD^+ dependent IDH; add $NADP^+$, the assay will detect $NADP^+$ dependent IDH; add both NAD^+ and $NADP^+$, the assay will detect total IDHs.

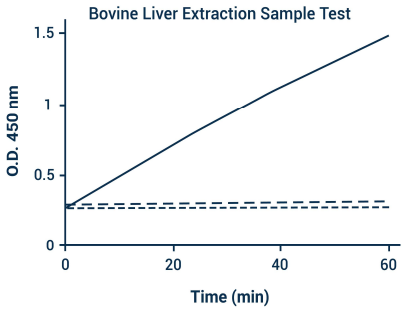
4. Incubate the mix for 3 min at 37 °C, then measure OD 450 nm in a microplate reader (A_0), incubate for another 30 min to 2 hr at 37 °C to measure OD 450 nm again (A_1), (Note: Incubation times will depend on the IDH activity in the samples). We recommend measuring the OD in a kinetic method (preferably every 1 – 5 min) and choose the period of linear range to calculate the IDH activity of the samples. The NADH Standard Curve can be read in Endpoint Mode (i.e., at the end of the incubation time).
5. **Calculation:** Subtract the 0 Standard values from all readings (standards and test samples). Plot the NADH Standard Curve. Calculate the IDH activity of the test samples: $\Delta OD = A_1 - A_0$, apply the ΔOD to the NADH standard curve to get B nmol of NAD(P)H generated by IDH during the reaction time ($\Delta T = T_2 - T_1$).

$$\textbf{IDH Activity} = \frac{B}{\Delta T \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

Where: **B:** the NAD (P)H amount from Standard Curve (in nmol).
T: the reaction time (in min).
V: the sample volume added into the reaction well (in ml).

Unit Definition: One unit IDH is the amount of enzyme that will generate 1.0 µmol of NADH or NADPH per min at pH 8 at 37 °C.





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Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		