

Isocitrate Dehydrogenase Activity Assay Kit

(Catalog #K756-100; 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 CO₂ 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⁺. BioVision'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	K756-100	Cap Code	Part Number
IDH Assay Buffer	25 ml	WM	K756-100-1
NAD ⁺ (lyophilized)	1 vial	Blue	K756-100-2
NADP ⁺ (lyophilized)	1 vial	Brown	K756-100-3
IDH Substrate (lyophilized)	1 vial	Red	K756-100-4
Developer (lyophilized)	1 vial	Purple	K756-100-5
IDH Positive Control (NADP ⁺)	20 µl	Green	K756-100-6
NADH Standard (0.5 µl mol, lyophilized)	1 vial	Yellow	K756-100-7

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 µl ddH₂O separately.
- Reconstitute the Developer with 0.9 ml of ddH₂O. Pipette up and down several times to completely dissolve the pellet into solution (**Don't vortex**).
- Reconstitute the NADH Standard with 50 µl ddH₂O 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:

- 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.
- Sample Preparations:** Tissues (50 mg) or cells (1 × 10⁶) 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 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).

- 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

- 40 µl IDH Assay Buffer
- 8 µl Developer
- 2 µl IDH Substrate
- 2 µl NAD⁺ or NADP⁺ or both (then use 38 µl Assay Buffer)*

Background Control Mix

- 42 µl IDH Assay Buffer
- 8 µl Developer

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.

- Incubate the mix for 3 min at 37 °C, then measure OD 450 nm in a microplate reader (A₀), incubate for another 30 min to 2 hr at 37 °C to measure OD 450 nm again (A₁), (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).
- 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: ΔOD = A₁ - A₀, apply the ΔOD to the NADH standard curve to get B nmol of NAD(P)H generated by IDH during the reaction time (ΔT = T₂ - T₁).

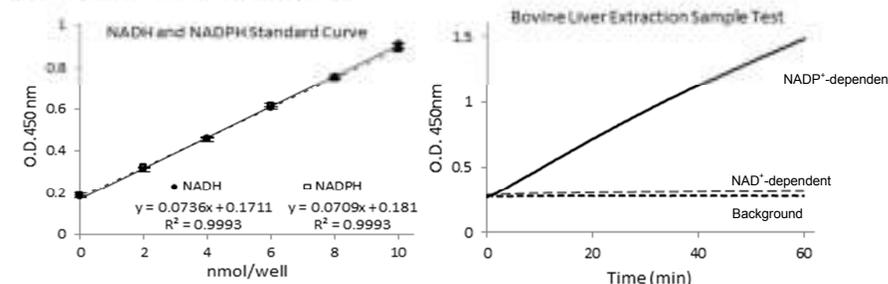
$$\text{IDH Activity} = \frac{B}{\Delta T \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

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.



VI. Related Products:

- NAD/NADH Quantification Kit
- Isocitrate Assay Kit
- Ethanol Assay Kit
- Lactate Assay Kit
- L-amino Acid Assay Kit
- Glycogen Assay Kit

- NADP/NADPH Quantification Kit
- ADP/ATP Ratio Assay Kit
- Pyruvate Assay Kit
- Lactate Assay Kit II
- Uric Acid Assay Kit
- Glutamate Kit

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
<p>Note# The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p>		