ab102528

Isocitrate Dehydrogenase Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Isocitrate Dehydrogenase activity in various samples
View kit datasheet: www.abcam.com/ab102528
(use www.abcam.cn/ab102528 for China, or www.abcam.co.jp/ab102528 for Japan)

This product is for research use only and is not intended for diagnostic use.

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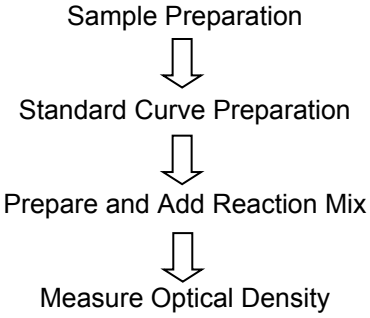
1. Overview

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^+.

Abcam'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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Isocitrate Assay Buffer	25 mL
NAD+	1 vial
Beta-NADP Stock	1 vial
IDH Substrate Mix	1 vial
Developer Solution II	1 vial
IDH Positive Control	20 µL
NADH Standard I	1 vial

* Store the kit at -20°C, protect from light.

- Allow Isocitrate Assay Buffer to warm to room temperature before use.
- Briefly centrifuge vials prior to opening.
- Read the entire protocol before performing the assay.
- All components are stable for up to 2 months at -20°C after reconstitution or freeze-thaw cycles (<5 times).

NAD^+, Beta-NADP STOCK, and IDH SUBSTRATE MIX: Reconstitute with 220 µl ddH_2O separately.

DEVELOPER SOLUTION II: Reconstitute with 0.9 ml of ddH_2O. Pipette up and down several times to completely dissolve the pellet into solution. DO NOT VORTEX.

NADH STANDARD I: Reconstitute with 50 µl ddH_2O to generate a 10 mM NADH stock solution.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Standard Curve Preparation:

a) Dilute 10 µl of the NADH Standard I solution with 90 µl of Assay Buffer to generate a 1 mM NADH standard.

b) 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.

c) 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 Preparation:

a. For tissue or cell samples: 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.

b. For serum samples: 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.

Notes:

a) For positive control (optional), add 2-5 µl positive control solution to wells and adjust to 50 µl with Assay Buffer.

b) 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 Step 3).

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	**Bkgd Control Mix**
Isocitrate Assay Buffer	38 µl	42 µl
Developer Solution II	8 µl	8 µl
IDH Substrate Mix	2 µl	---
NAD^+/Beta-NADP Stock	2 µl	---

Notes:

a) If using both NAD^+ and Beta-NADP Stock, add 2 µl of each and use only 36 µl of Assay Buffer.

b) Add NAD^+, the assay will detect NAD^+ dependent IDH; add Beta-NADP Stock, the assay will detect NADP+ dependent IDH; add both NAD^+ and Beta-NADP Stock, the assay will detect total IDHs.

c) Note: Use Beta-NADP Stock for positive control

4. Incubate the mix for 3 min at 37 °C, then measure OD_{450nm} in a microplate reader (A_0). Incubate for another 30 min to 2 hr at 37 °C to measure OD_{450nm} again (A_1).

Notes:

a) Incubation times will depend on the IDH activity in the samples.

b) 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. Data Analysis

Subtract the zero Standard values from all readings (standards and test samples).

Plot the NADH Standard Curve. Calculate the IDH activity of the test samples:

$$\mathbf{\Delta OD = A_1 - A0,}$$

Apply the ΔOD to the NADH standard curve to get B nmol of NAD(P)H generated by IDH during the reaction time ($\Delta T = T_2 - T_1$).

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

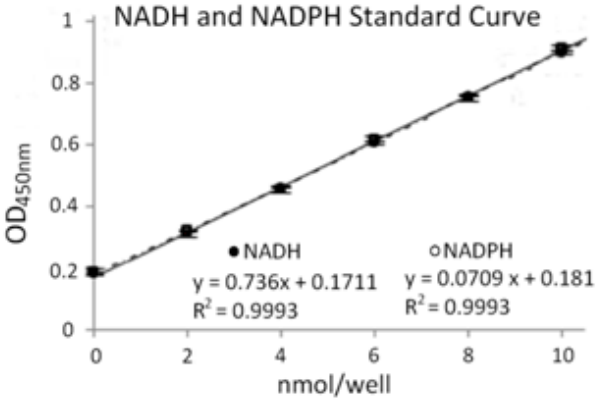
Where:

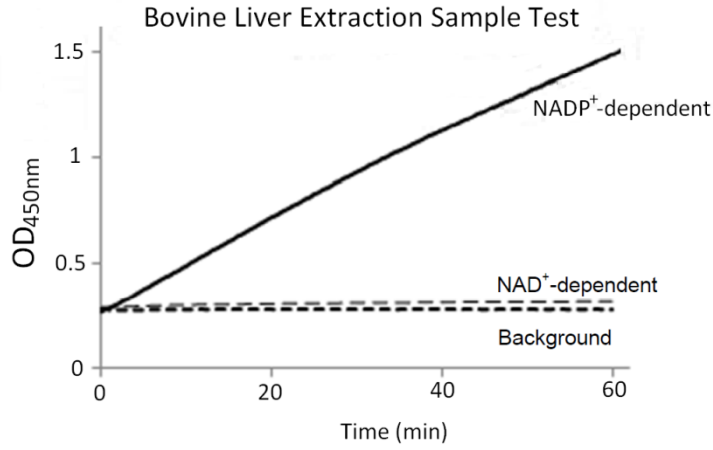
B is the NAD(P)H amount from Standard Curve (in nmol).

T is the reaction time (in min).

V is 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


NADH and NADPH Standard Curve
OD450nm
nmol/well
NADH
NADPH
y = 0.736x + 0.1711
R2 = 0.9993
y = 0.0709 x + 0.181
R2 = 0.9993


Bovine Liver Extraction Sample Test
OD450nm
Time (min)
NADP+-dependent
NAD+-dependent
Background

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to: www.abcam.com/contactus

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