Version 1 Last updated 4 June 2020

ab273418 Isopropanol Dehydrogenase Activity Assay Kit (Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273418
(use https://www.abcam.cn/ab273418 for china, or
https://www.abcam.co.jp/ab273418 for Japan)

For the determination of Isopropanol Dehydrogenase activity in various samples.

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

Copyright © 2020 Abcam. All rights reserved

Table of Contents

1. Overview 1
2. Protocol Summary 2
3. Precautions 3
4. Storage and Stability 3
5. Limitations 4
6. Materials Supplied 4
7. Materials Required, Not Supplied 5
8. Technical Hints 5
9. Reagent Preparation 6
10. Sample Preparation 7
11. Standard Curve 8
12. Assay Procedure 9
13. Calculations 10
14. Typical Data 11
15. FAQ / Troubleshooting 12
16. Notes 13

Copyright © 2020 Abcam. All rights reserved

1. Overview

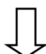
Isopropanol Dehydrogenase Activity Assay Kit (Colorimetric) (ab273418) provides a convenient tool for sensitive detection of the Isopropanol Dehydrogenase (ADH1-NADP) in a variety of samples (bacterial, yeast or protozoan, animal tissues and cells). In this assay, Isopropanol gets converted to Acetone and NADPH in the presence of NADP+. This results in the development of color in the presence of the developer. This color 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.

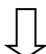
2. Protocol Summary

Prepare all reagents, samples and positive controls.

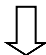
Prepare standards.



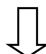
Add all samples to the appropriate wells.



Add Reaction Mix and Background Control Mix to the appropriate wells.



Measure absorbance in kinetic mode for 10-120 mins at 37°C.



Determine Isopropanol Dehydrogenase activity using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
ADH1-NADP Assay Buffer	25 ml	-20°C
ADH1-NADP Developer Lyophilized	1 vial	-20°C
ADH1-NADP Positive Control	100 µl	-20°C
ADH1-NADP Substrate	1 ml	-20°C
NADPH Standard Lyophilized	1 vial	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

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

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

– Briefly centrifuge small vials at low speed prior to opening.

9.1 **ADH1-NADP Assay Buffer:**

Ready to use as supplied. Warm to room temperature before use. Store at either 4°C or -20°C.

9.2 **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.

9.3 **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.

9.4 **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.

9.5 **ADH1-NADH Substrate:**

Ready to use as supplied. Store at -20°C. Keep on ice during use.

10. Sample Preparation

10.1 Homogenize ~10-50 mg tissues or ~1 x 10^6 cells in 200 µl ice-cold Assay Buffer.

10.2 Centrifuge at 13,000 *x g*, 4°C for 10 mins and collect the supernatant.

10.3 Add 2 - 50 µl supernatant per well and adjust the volume to 50 µl with the Assay Buffer.

10.4 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).

10.5 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.

Δ **Note:** For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

Δ **Note:** For samples with significant background, prepare parallel sample well(s) as background controls (BCs).

11. Standard Curve

– Keep standards on ice while in use.

11.1 Dilute 1 mM NADPH Standard 1:5 to 0.2 mM NADPH by adding 20 µl NADPH Standard to 80 µl Assay Buffer.

11.2 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.

11.3 Adjust the final volume to 50 µl with ADH1-NADP Assay Buffer.

Standard #	0.2 mM NADPH Standard (µL)	ADH1-NADP Assay Buffer (µL)	NADPH (nmol/well)
1	0	50	0
2	10	40	2
3	20	30	4
4	30	20	6
5	40	10	8

12. Assay Procedure

– Keep on ice while in use.

12.1 **Reaction Mix:**
Prepare enough reagents for the number of assays to be performed. For each well, prepare 100 µl of the Reaction mix:

	Reaction mix (µl)	***Background Control mix (µl)**
ADH1-NADP Assay Buffer	82	92
ADH1-NADP Developer	8	8
ADH1-NADP Substrate	10	---

12.2 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.

12.3 Start measuring absorbance immediately at 450 nm in a kinetic mode for 10-120 mins at 37°C.

Δ 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 (T1 and T2) 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).

13. Calculations

13.1 Subtract 0 Standard reading from all readings.
13.2 Plot the NADPH Standard Curve.
13.3 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 $\Delta BC = BC_2 - BC_1$.

13.4 Apply the ΔOD to the NADPH Standard Curve to get B nmol of NADPH generated during the reaction time ($\Delta T = T_2 - T_1$).

$$ADH1\ NADH\ activity = \frac{B}{(\Delta T\ x\ V)}\ X\ D = nmol/min/ml = mU/ml$$

B = NADPH amount from the Standard Curve (nmol)
ΔT = Reaction time ($T_2 - T_1$) (mins)
V = Sample volume initially added to reaction well (ml)
D = Dilution factor (D=1 for undiluted samples)

Unit Definition: One unit of ADH1-NADP is the amount of enzyme that generates 1.0 µmol of NADPH per min at pH 8.0 at 37°C.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

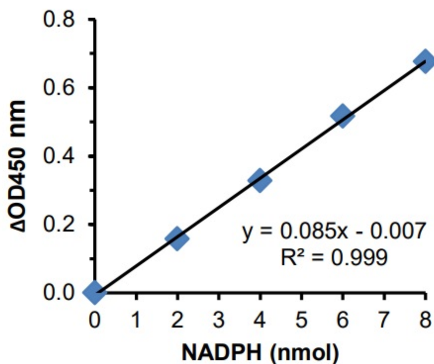


Figure 1. NADPH standard curve.

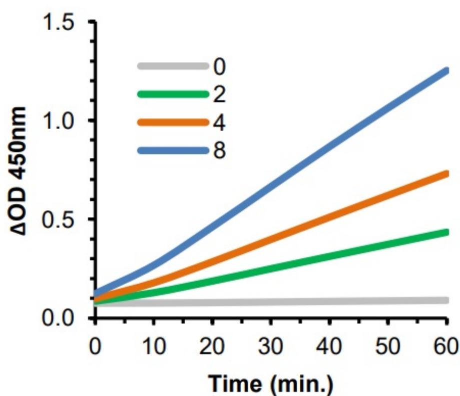


Figure 2. ADH1-NADP Positive Controls (µl/assay).

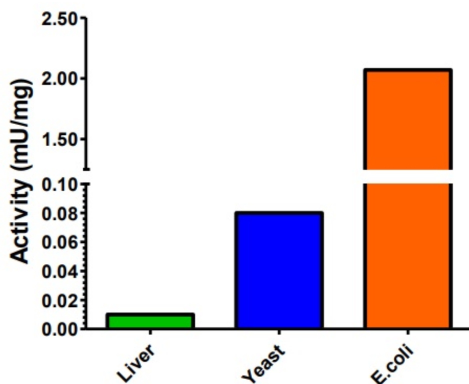


Figure 3. ~5 mg of bovine liver, yeast and E.coli cell pellet were homogenized as described in the kit protocol. Serial dilutions were tested to ensure the readings were within the linear range of the Standard Curve. ADH1-NADP activities (mU/mg) of samples were measured and calculated as in the kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

Copyright © 2020 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)