ab273327 – 15-PGDH Activity Assay Kit (Fluorometric)

For the oxidization of substrate forming intermediates and NAD.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab273327

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
15-PGDH Assay Buffer	25 mL	-20°C
Developer Solution X	1 vial	-20°C
15-PGDH Positive Control	100 µl	-20°C
15-PGHD Substrate	100 µl	-20°C
NADH Standard II	1 vial	-20°C
PicoProbe I	0.4 ml	-20°C

PLEASE NOTE: Developer Solution X as previously labelled as 15-PGDH Developer (Lyophilized), and PicoProbe I as Probe (in DMSO), and NADH Standard II as NADH Standard (Lyophilized). The composition has not changed.

Materials Required, Not Supplied

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

- 96-well white opaque plate with flat bottom.
- Multi-well spectrophotometer (fluorescence plate reader)
- Multi-channel pipette

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Developer Solution X: Reconstitute with 220 µl of Assay Buffer. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.

NADH Standard II: Reconstitute with 200 µl dH2O to generate 1 mM (1 nmol/µl) NADH Standard II solution. Aliquot and store at –20°C. Use within two months. Keep on ice while in use.

15-PGDH Positive Control: Aliquot and store at -20°C. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.
Note: *Positive Control may saturate depending on the plate reader used. If needed, the positive control can be titrated down.*

Sample Preparation

1. Homogenize tissue (~10 mg) or cells (1 x 106) with 100 µl ice-cold 15-PGDH Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 x g, 4°C for 5 min and collect supernatant.
2. Use ammonium sulfate precipitation method to remove small molecules that could interfere with the assay: Aliquot tissue samples (100 µl) to a clean centrifuge tube, and add 200 µl saturated (4.32 M) ammonium sulfate and place sample on ice for 30 min.
3. Spin down samples at 10,000 x g at 4°C for 10 mins, discard the supernatant, and resuspend the pellet back to the original volume (100 µl) using 15-PGDH Assay Buffer.
4. Add 2-50 µl into desired well(s) in a 96-well white plate. For 15-PGDH Positive Control, add 2-20 µl of 15-PGDH Positive Control into desired well(s). Adjust the volume of Positive Control and sample wells to 50 µl/well with 15-PGDH Assay Buffer.
   Notes:
   a) For unknown samples, we suggest doing pilot experiment and testing several amounts of sample to ensure the readings are within the Standard Curve range.
   b) If sample has high background, prepare parallel sample well(s) as sample background control.

NADH Standard Curve:

1. Dilute NADH Standard II to 20 µM (20 pmol/µl) by adding 20 µl of 1 mM NADH Standard II to 980 µl of 15-PGDH Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of 20 µM NADH Standard II into a series of wells in a 96-well plate to generate 0, 40, 80, 120, 160 and 200 pmol/well of NADH Standard II.
2. Adjust the volume to 50 µl/well with 15-PGDH Assay Buffer.

Reaction Mix:

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containin:

Item	Reaction Mix	*Background Control Mix
15-PGDH Assay Buffer	46 µl	47 µl
PicoProbe I	1 µl	1 µl
Developer Solution X	2 µl	2 µl
15-PGDH Substrate	1 µl	-

2. Mix and add 50 µl of Reaction Mix into each well containing Standards, Positive Control, and Samples. Mix well.
   ***Note:** For samples having background, add 50 µl of Background Control Mix to sample background control well(s).

Measurement

- Measure fluorescence (Ex/Em = 535/587 nm) immediately in kinetic mode for 10-40 min. at 37°C.
  ΔNote: Incubation time depends on the 15-PGDH activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (t1 and t2) in the linear range to calculate the 15-PGDH activity of the samples. The NADH Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

Calculation

- Subtract 0 Standard reading from all readings. Plot the NADH Standard curve. If sample background control reading is significant, subtract the sample background control reading from sample reading.
- Calculate the 15-PGDH activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$. Apply ΔRFU to NADH Standard Curve to get B pmol of NADH generated by 15-PGDH during the reaction time ($\Delta t = t_2 - t_1$).

Sample 15-PGDH Activity = B/(Δt X V) x D = pmol/min/µl = µU/µl = mU/ml

Where: **B** = NADH amount in the sample well from Standard Curve (pmol)
Δt = Reaction time (min.)
V = Sample volume added into the reaction well (µl)
D = Dilution factor (D=1 if undiluted) 15-PGDH Activity in samples can also be expressed in mU/mg of protein.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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