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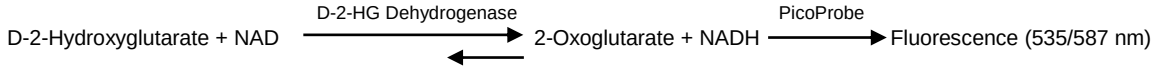
PicoProbe™ D-2-Hydroxyglutarate Dehydrogenase Assay Kit (Fluorometric)

(Catalog # K248-100; 100 assays; Store at -20°C) *2/16*

I. Introduction:

D-2-Hydroxyglutarate Dehydrogenase (D2HGDH: EC 1.1.99.2) is a mitochondrial enzyme which catalyzes the conversion of D-2-Hydroxyglutarate into 2-Oxoglutarate. In humans, mutation of D2HGDH gene could reduce D2HGDH activity which will cause the D-2-Hydroxyglutarate accumulation in body fluids. Increased D-2-Hydroxyglutarate level in body fluids leads to D-2-Hydroxyglutaric Aciduria, a neurometabolic disorder characterized as developmental delay, epilepsy, hypotonia, and dysmorphic features. BioVision's PicoProbe™ D-2-Hydroxyglutarate Dehydrogenase Assay kit provides a quick and easy method for monitoring D2HGDH activity in a wide variety of samples. In this assay, D2HGDH converts D-2-Hydroxyglutarate into 2-Oxoglutarate and NADH, which further reduces PicoProbe™ to generate an intense fluorescent product (Ex/Em = 535/587 nm). This kit is simple, sensitive and high-throughput adaptable and can detect as low as 4 µU/µl of D2HGDH activity.



II. Applications:

- Measurement of D2HGDH activity in purified enzyme preparations, tissues and cells

III. Sample Type:

- Animal tissues: liver, kidney, heart, brain, etc.
- Cell culture: adherent or suspension cells

IV. Kit Contents:

Components	K248-100	Cap Code	Part Number
D2HGDH Assay Buffer	20 ml	WM	K248-100-1
PicoProbe™ (in DMSO)	0.2 ml	Blue	K248-100-2
D2HGDH Substrate	0.4 ml	Orange	K248-100-3
D2HGDH Developer	1 Vial	Red	K248-100-4
D2HGDH Positive Control	1 Vial	Purple	K248-100-5
NADH Standard	1 Vial	Yellow	K248-100-6

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **D2HGDH Assay Buffer:** Bring to room temperature before use. Store at 4°C or -20°C.
- **PicoProbe™ :** Before use, thaw at room temperature. Store at -20°C. Use within two months.
- **D2HGDH Substrate:** Store at -20°C, stable for 6 months.
- **D2HGDH Developer:** Reconstitute with 220 µl Assay Buffer. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **D2HGDH Positive Control:** Reconstitute with 100 µl Assay Buffer and mix thoroughly. Aliquot and store at -70°C. Avoid freeze/thaw. Use within two months. Keep on ice while in use.
- **NADH Standard:** Reconstitute with 200 µl dH_2O to generate 1 mM (1 nmol/µl) NADH Standard solution. Aliquot and store at –20°C. Use within two months. Keep on ice while in use.

VII. D2HGDH Activity Assay Protocol:

1. **Sample Preparation:** Homogenize tissue (~10 mg) or cells (1 x 10^6) with 100 µl ice cold D2HGDH Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 X g, 4°C for 5 min. and collect supernatant. Dilute the supernatant ~10 fold in Assay Buffer and add 1-50 µl into desired well(s) in a 96-well plate. For Positive Control, dilute D2HGDH Positive Control 10 times with D2HGDH Assay Buffer just before use and add 2-20 µl of diluted D2HGDH Positive Control into desired well(s). Adjust the volume of Positive Control and sample wells to 50 µl/well with D2HGDH Assay Buffer.

   Notes:

   a. For unknown samples, we suggest doing pilot experiment and testing several amounts of D2HGDH to ensure the readings are within the Standard Curve range.

   b. If sample has high background due to the interference from the NADH in the samples, we recommend to use 3.2 mM ammonium sulfate (final concentration; BioVision Cat. No. 7096) to precipitate the protein, discard the supernatant, resuspend the protein in the original volume in D2HGDH Assay Buffer, and prepare parallel sample well(s) as sample background control.

   c. Don't store the diluted D2HGDH Positive Control.

2. **NADH Standard Curve:** Dilute NADH Standard to 50 µM (50 pmol/µl) by adding 10 µl of 1 mM NADH Standard to 240 µl of D2HGDH Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of 50 µM NADH Standard into a series of wells in a 96-well plate to generate 0, 100, 200, 300, 400 and 500 pmol/well of NADH Standard. Adjust the volume to 50 µl/well with D2HGDH Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing Mix. Add 50 µl of Reaction Mix to each well containing Standards, Positive Control, and samples. Mix well.

	Reaction Mix	*Background Control Mix
D2HGDH Assay Buffer	45 µl	47 µl
PicoProbe™	1 µl	1 µl
D2HGDH Developer	2 µl	2 µl
D2HGDH Substrate	2 µl	----

* For samples having high background, add 50 µl of Background Control Mix to sample background control well(s).

4. **Measurement:** Measure fluorescence (Ex/Em = 535/587 nm) immediately in kinetic mode for 10-40 min. at 37°C.

   Note: Incubation time depends on the D2HGDH activity in the samples. We recommend measuring the reaction progress in kinetic mode, and choosing two time points (T_1 and T_2) in the linear range to calculate the D2HGDH activity of the samples. The NADH Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

5. **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 D2HGDH 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 D2HGDH during the reaction time ($\Delta T = T_2 - T_1$).

Sample D2HGDH Activity = B/(ΔT X V) x D = pmol/min/µl = µU/µl = mU/ml

Where: **B** is NADH amount in the sample well from Standard Curve (pmol)
ΔT is reaction time (min.)
V is sample volume added into the reaction well (µl)
D is dilution factor

D2HGDH Activity in samples can also be expressed in mU/mg of protein.

Unit Definition: One unit of D2HG Dehydrogenase is the amount of enzyme that generates 1.0 µmol of NADH per min. at pH 8.0 at 37°C.

(a) (b) (c)

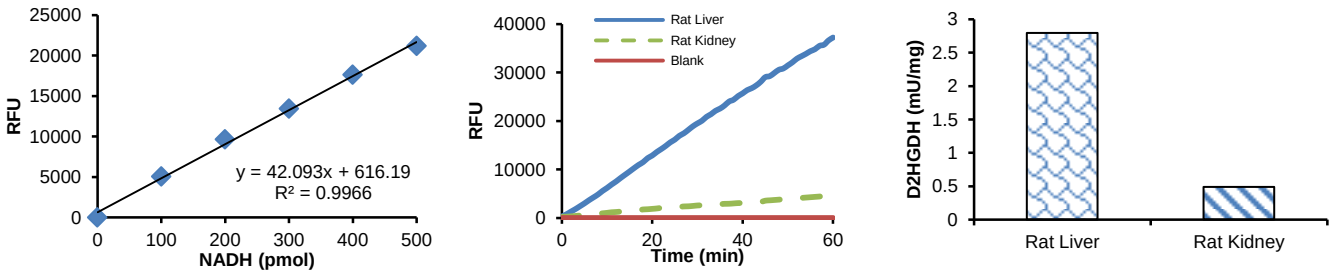


Figure: (a) NADH Standard Curve. (b) Kinetic measurement of D2HGDH activity in various samples. (c) D2HGDH specific activity was calculated in lysates prepared from Rat Liver (5 µg) and Rat Kidney (4 µg). Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Active D-2-Hydroxyglutaratate Dehydrogenase (P1001)
Alpha-Ketoglutarate Assay Kit (K677)
Pyruvate Dehydrogenase Activity Assay Kit (K679)
PicoProbe™ NADH Assay Kit (K338)
Glucose-6-Phosphate Dehydrogenase Activity Assay Kit (K757)
Phosphoglucomutase Assay Kit (K774)
PicoProbe™ D-Lactate Fluorometric Assay Kit (K668)
Glucose Dehydrogenase Activity Assay Kit (K786)
D-2-Hydroglutarate (D2HG) Assay Kit (K213)
Pyruvate Colorimetric/Fluorometric Assay Kit (K609)
Triose Phosphate Isomerase Assay Kit (K670)
PicoProbe™ NADPH Assay Kit (K349)
Glucose-6-Phosphate Assay Kit (K657)
Phosphoglucose Isomerase Assay Kit (K775)
Glucose-1-Phosphate Assay Kit (K697)

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