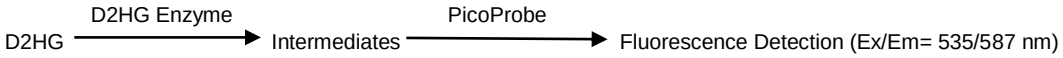
PicoProbe™ D-2-Hydroxyglutarate (D2HG) Assay Kit

7/16

(Catalog #K970-100; 100 assays; Store at -20°C)

I. Introduction:

In eukaryotic cells, Isocitrate Dehydrogenase (IDH1, IDH2 and IDH3) catalyzes the interconversion of Isocitrate and α-Ketoglutarate. In human cancers, IDH mutations can cause a gain-of-function, which reduces its affinity for isocitrate and facilitates the conversion of α-ketoglutarate to D-2-Hydroxyglutarate in the presence of NADP. D-2-Hydroxyglutarate (D2HG) is present at low level in normal cells and tissues, but is significantly elevated in metabolic diseases and various cancers. Therefore, detection of elevated D2HG is an important biomarker for early diagnosis, prognosis and the development of therapeutic strategies against these maladies. BioVision's PicoProbe™ D-2-Hydroxyglutarate Assay Kit provides a convenient method to detect low levels of D2HG in biological samples. In this assay, D-2-Hydroxyglutarate is oxidized to α-Ketoglutarate in the presence of D2HG Enzyme Mix and Substrate Mix. The intermediate reduces the PicoProbe™ to generate fluorescence which is directly proportional to the amount of D2HG present in the samples. This assay kit is fast, sensitive, easy to use and high-through adaptable. It can measure D-2-Hydroxyglutarate levels less than 0.1 µM in various samples.



II. Application:

- Measurement of D2HG level in various biological fluids

III. Sample Type:

Biological Samples: Serum, Plasma, etc.

IV. Kit Contents:

Components	K970-100	Cap Code	Part Number
D2HG Assay Buffer	20 ml	WM	K970-100-1
PicoProbe™ (in DMSO)	200 µl	Blue	K970-100-2
D2HG Enzyme	1 vial	Green	K970-100-3
D2HG Developer	1 vial	Red	K970-100-4
D2HG Standard	1 vial	Yellow	K970-100-5

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

Store kit at -20°C, protected from light. Bring the D2HG Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Read the entire protocol before the assay.

VII. Reagent Preparation and Storage Conditions:

- **PicoProbe™ (in DMSO):** Ready to use as supplied. Warm to room temperature before use. Store at -20°C.
- **D2HG Enzyme and D2HG Developer:** Reconstitute with 220 µl D2HG Assay Buffer. Pipette up and down to dissolve completely. Buffer. Avoid repeated freeze/thaw. Aliquot and store at -20°C. Keep on ice while in use. Stable for 2 months at -20°C.
- **D2HG Standard:** Reconstitute with 50 µl dH_2O to generate 100 mM (100 nmol/µl) D-2-Hydroxyglutarate Standard solution. Keep on ice while in use. Store at -20°C. Use within 2 months.

VIII. PicoProbe™ D2HG Assay Protocol:

1. **Sample Preparation:** Serum or plasma samples need to be spin filtered using a 10 kD spin column (BioVision Cat# 1997-25) to remove enzymatic activity. Use the flow through for measurement. Add the same volume (0-45 µl) of each sample into three wells of a 96-well white plate with flat bottom. Label these wells as Sample Background, Sample and Spiked Sample.
2. **Standard Curve Preparation:** Dilute D-2-Hydroxyglutarate standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM D-2-Hydroxyglutarate Standard to 990 µl D2HG Assay Buffer and mix well. Dilute 1 mM to 10 µM (10 pmol/µl) by adding 10 µl to 990 µl D2HG Assay Buffer, mix well. Add 0, 4, 8, 12, 16, 20 µl of 10 µM D-2-Hydroxyglutarate into a 96-well white plate to generate 0, 40, 80, 120, 160, 200 pmol/well standards. Adjust volume to 50 µl per well with Assay Buffer.
3. **Internal Standard:** Add 5 µl of 10 µM D2HG standard to one of three sample wells ("Spiked Sample": 50 pmol D-2-Hydroxyglutarate + Sample). The Spiked Sample is used as an internal standard to correct for sample interference. Adjust final volume of all wells to 50 µl with D2HG Assay Buffer.
4. **Reaction Mix:** Mix enough reagents for the number of assays (Samples, Spiked Samples and all Standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	*Background Control Mix
D2HG Assay Buffer	45.7 µl	47.7 µl
D2HG Enzyme	2 µl	0 µl
D2HG Developer	2 µl	2 µl
PicoProbe™	0.3 µl	0.3 µl

Note: * For samples having background, add 50 µl of the background control mix to sample background control well(s). Add 50 µl of the Reaction Mix to each well containing the Standards, Samples and Spiked Samples. Mix well.

5. **Measurement:** Incubate for 60 min at 37°C and measure the fluorescence at Ex/Em = 535/587 nm in a micro plate reader.
6. **Calculation:** Subtract the Reagent Background reading from all standard and sample readings. Correct for any sample interference by subtracting the fluorescence of the samples from the fluorescence of the internal standards (sample + standard). Determine the D-2-Hydroxyglutarate amount (X) in the sample wells based on the following equation:

$$\text{D2HG amount (pmol)} = \left(\frac{(\text{RFU}_{\text{sample (corrected)}})}{(\text{RFU}_{\text{(sample + 50 Corrected)}})-(\text{RFU}_{\text{sample(corrected)}})}\right) * 50$$

The D-2-Hydroxyglutarate concentration in the sample:

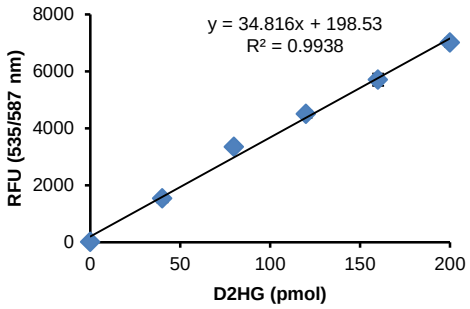
C = X/V x D = pmol/µl = µmol/l or µM

Where **X** = the amount of D-2-Hydroxyglutarate (nmol) from the calculation above
V = the sample volume added into reaction well (µl)
D = Sample Dilution Factor
50 = Amount spiked in sample well (50 pmol)

D-2-Hydroxyglutarate MW = 192.08

Sample D-2-Hydroxyglutarate concentration can also be expressed in pmol/mg or µmol/g of sample

(a)



(b)

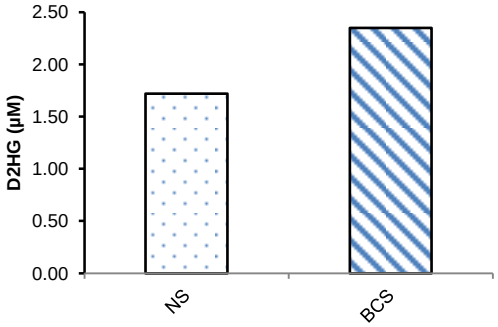


Figure: (a) D2HG Standard Curve generated using this kit. (b) Measurement of D-2-Hydroxyglutarate in Human Serum from pooled healthy people (NS: 10 µl) and human serum from Breast Cancer patient (BCS: 10 µl). Samples were filtered using 10 kD Spin Column, and Spiked Samples with D2HG (50 pmol), and assayed following the kit protocols.

IX. RELATED PRODUCTS:

D-2-Hydroxyglutarate (D2HG) Colorimetric Assay Kit (K213)
Malate Colorimetric Assay Kit (K637)
Pyruvate Colorimetric /Fluorometric Assay Kit (K609)
Citrate Colorimetric/ Fluorometric Assay Kit (K655)
Citrate Synthase Activity Colorimetric Assay Kit (K318)
Succinate (Succinic Acid) Colorimetric Assay Kit (K649)
α-Ketoglutarate Colorimetric/Fluorometric Assay Kit (K677)
Active D-2-Hydroxyglutaratate Dehydrogenase (D2HGDH) (P1001)
PicoProbe™ D2HG Dehydrogenase Fluorometric Assay Kit (K248)
Fumarate Colorimetric Assay Kit (K633)
Succinate Dehydrogenase Activity Assay Kit (K660)
Oxaloacetate Colorimetric/Fluorometric Assay kit (K659)
Isocitrate Colorimetric Assay Kit (K656)
Isocitrate Dehydrogenase Activity Assay Kit (K756)
Aconitase Activity Colorimetric Assay Kit (K716)

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