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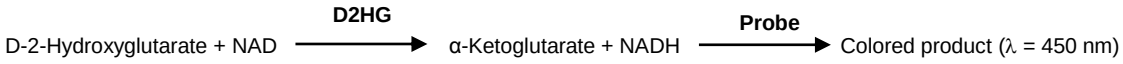
D-2-Hydroxyglutarate (D2HG) Assay Kit (Colorimetric)

rev 7/19

(Catalog #K213-100; 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, an IDH mutation causes 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 in low level in normal cells and tissues, but is significantly elevated in metabolic diseases and various cancers. Therefore, detection of elevated D2HG is important for early diagnosis, prognosis and the development of therapeutic strategies against these maladies. BioVision's D-2-Hydroxyglutarate Assay Kit provides a convenient method to detect D2HG in biological samples. In this assay, D2HG is oxidized to α-Ketoglutarate in the presence of D2HG Enzyme and Substrate Mix. The intermediate reduces the probe to a colored product with strong absorbance at 450 nm. The absorbance is proportional to the amount of D2HG present in the samples. The assay kit is fast, sensitive, easy to use and high-through adaptable. It can measure D2HG levels less than 10 µM in various samples.



II. Application:

- Measurement of D2HG level in various cell, tissues or biological fluids.

III. Sample Type:

Adherent or suspension cells: e.g. 3T3, HepG2, Jurkat cells.
Tissues: e.g. Rat Liver, Rat Kidney, etc.
Biological samples: Urine.

IV. Kit Contents:

Components	K213-100	Cap Code	Part Number
D2HG Assay Buffer	20 ml	WM	K213-100-1
D2HG Enzyme	1 vial	Green	K213-100-2
D2HG Substrate Mix	1 vial	Red	K213-100-3
D2HG Standard	1 vial	Yellow	K213-100-4

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

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

VII. Reagent Preparation and Storage Conditions:

- **D2HG Assay Buffer:** Allow the D2HG Assay Buffer to warm to room temperature (RT) prior to use.
- **D2HG Enzyme:** Reconstitute with 220 µl D2HG Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Stable for 2 months.
- **D2HG Substrate Mix:** Dissolve with 220 µl dH_2O. Pipette up and down to dissolve completely. Stable for 2 months at -20°C.
- **D2HG Standard:** Reconstitute with 50 µl dH_2O to generate 100 mM (100 nmol/µl) D2HG Standard solution. Keep on ice while in use. Store at -20°C. Use within 2 months.

VIII. D2HG Assay Protocol:

1. **Sample Preparation:** Serum and Plasma samples can be measured directly. Urine Samples need to be spun down at 10,000 x g for 5 min at RT to collect the supernatant. Tissue (~10 mg) or cells (~1 x 10^7) should be rapidly homogenized with 100 µl ice cold D2HG Assay Buffer for 10 min on ice. Centrifuge at 10,000 x g, 4ºC for 5 min, collect the supernatant. Add the same volume (0-45 µl) of each Sample into three wells of a 96 well clear plate.
   Note: If the Samples are not clear, they need to be spin filtered using either a 0.22 µm filter or a 10 kD spin column (BioVision Cat# 1997-25) with the added benefit of removal of possible interfering enzyme activity to remove the insoluble components. Use the flow through for the assay.
2. **Standard Preparation:** Dilute the 100 mM D2HG Standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM D2HG Standard solution to 990 µl D2HG Assay Buffer and mix well.
3. **Internal Standard:** Add 5 µl of 1 mM D2HG Standard to one of three Samples defined as: **Spiked Sample** (5 nmol D-2-Hydroxyglutarate + Sample); **Sample**; and **Sample Background**. The Spiked Sample is used as an Internal Standard to correct for any Sample interference. Adjust final volume of all wells to 50 µl with D2HG Assay Buffer.

4. Reaction Mix Preparation: Mix enough reagents for the number of assays (Samples and Standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	*Background Control Mix
D2HG Assay Buffer	46 µl	48 µl
D2HG Enzyme	2 µl	0 µl
D2HG Substrate Mix	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standards and Samples. Mix well.
**Note: ***For Samples having Background, add 50 µl of the Background Control mix to Sample Background well(s) and use these values for Sample correction.

5. **Measurement:** Incubate the plate for 60 min at 37°C and measure $OD_{450\ nm}$.
6. **Calculation:** Subtract the Sample Background reading from its paired Sample reading to get Sample Corrected reading. Determine the D2HG amount in the Sample wells (X) based on the following equation:

$$\text{D2HG amount (nmol)} = \left(\frac{(OD_{\text{sample (corrected)}})}{(OD_{\text{(spiked sample)}})-(OD_{\text{sample)}})}\right) * 5$$

The D2HG concentration in the Sample is:

C = X/V x D = nmol/µl = mmol/l or mM

Where: **X** = Amount of D2HG from the calculation above (nmol)

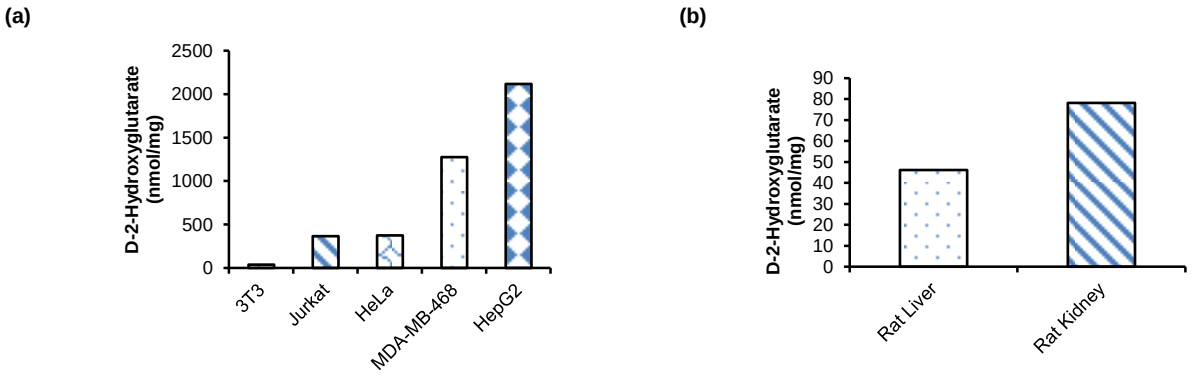
V = Sample volume added into reaction well (µl)

D = Sample Dilution Factor

5 = Amount spiked in Sample well (5 nmol)

D2HG MW = 192.08

Sample D2HG concentration can also be expressed in nmol/mg or µmol/g of Sample



Figures: **(a)** Measurement of D2HG in different cell lysates: 3T3 (80 µg), Jurkat (12 µg), HeLa (15 µg), MDA-MB-468 (6 µg), and HepG2 (2 µg). **(b)** Measurement of D2HG in rat liver lysate (120 µg) and rat kidney lysate (240 µg). Assays were performed following the protocol.

IX. Related Products:

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)
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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