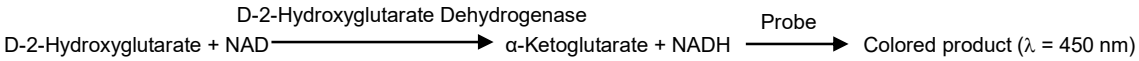
D-2-Hydroxyglutarate (D2HG) Assay Kit (Colorimetric)

(Catalog #BN00490; 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 at 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. Assay Genie's D-2-Hydroxyglutarate Assay Kit provides a convenient method to detect D2HG in biological samples. In this assay, D-2-Hydroxyglutarate 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. This absorbance is 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 10 µM in various samples.



II. Application:

- Measurement of D2HG level in various cell/tissues/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	BN00490	Cap Code	Part Number
D2HG Assay Buffer	20 ml	WM	BN00490-1
D2HG Enzyme	1 vial	Green	BN00490-2
D2HG Substrate Mix	1 vial	Red	BN00490-3
D2HG Standard	1 vial	Yellow	BN00490-4

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. 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:

- **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) D-2-Hydroxyglutarate 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 room temperature 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 minutes 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 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 measurement.
- For unknown samples, we suggest testing several doses to ensure that the readings do not exceed the signal from the External standard (see below), dilute samples if the OD450 nm is more than 1.4.

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.
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 sample interference. Adjust final volume of all wells to 50 µl with D2HG Assay Buffer.

4. **External standard:** Add 0 and 20 µl of 1 mM D2HG standard to two wells defined as: Reagent Background and External Standard. Adjust final volume of the well(s) to 50 µl with D2HG Assay Buffer.
5. **Reaction Mix:** Mix enough reagents for the number of assays (samples and standards, including the internal and external 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

Note: * For samples having background, add 50 µl of the background control mix to sample background control well(s) and use these values for sample correction.
Add 50 µl of the Reaction Mix to each well containing the Standards and test samples. Mix well.

6. **Measurement:** Incubate for 60 min at 37°C and measure $OD_{450\ nm}$.
7. **Calculation:** Subtract the Reagent Background reading from all standard and sample readings. Correct for any sample interference by subtracting the absorbance of the samples from the absorbance of the internal standards (sample + standard). Determine the D-2-Hydroxyglutarate amount (X) in the sample wells based on the following equation:

$$\text{D-2-Hydroxyglutarate amount (nmol)} = \left(\frac{(OD_{\text{sample (corrected)}})}{(OD_{\text{(Spiked Sample)}})-(OD_{\text{sample(corrected)}})}\right) * 5$$

The D-2-Hydroxyglutarate concentration in the sample:

C = X/V x D = nmol/µl = mmol/l or mM

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

5 = Amount spiked in sample well (5 nmol)

D-2-Hydroxyglutarate MW = 192.08

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

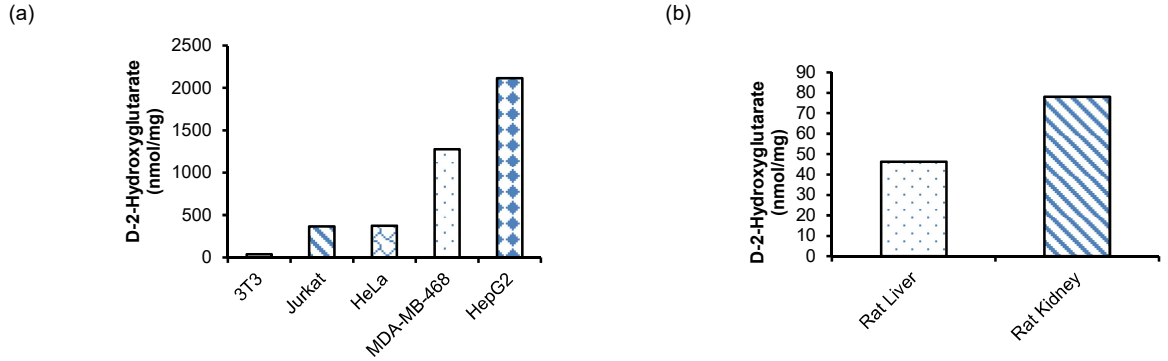


Figure: (a) Measurement of D-2-Hydroxyglutarate in different cell lysates: 3T3 (80 µg), Jurkat (12 µg), HeLa (15 µg), MDA-MB-468 (6 µg), and HepG2 (2 µg). (b) Measurement of D-2-Hydroxyglutarate in rat liver lysate (120 µg) and rat kidney lysate (240 µg). Assays were performed following the protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.