ab211070 – D 2 Hydroxyglutarate Assay Kit (Colorimetric)

For the measurement of D2HG in various cells, tissues or biological fluids.
For research use only - not intended for diagnostic use.
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab211070

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Read the entire protocol before performing the assay. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer XXXII/D2HG Assay Buffer	20 mL	-20°C
D2HG Enzyme	1 vial	-20°C
Developer Solution III/D2HG Substrate Mix	1 vial	-20°C
D2HG Standard	1 vial	-20°C

Materials Required, Not Supplied

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

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

Reagent Preparation

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

Assay Buffer XXXII/D2HG Assay Buffer: Allow the Assay Buffer XXXII/D2HG Assay Buffer to warm to room temperature (RT) prior to use.

D2HG Enzyme: Reconstitute with 220 µl Assay Buffer XXXII/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.

Developer Solution III/D2HG Substrate Mix: Dissolve with 220 µl dH2O. Pipette up and down to dissolve completely. Stable for 2 months at -20°C.

D2HG Standard: Reconstitute with 50 µl dH2O to generate 100 mM (100 nmol/µl) D2HG Standard solution. Keep on ice while in use. Store at -20°C. Use within 2 months.

Assay Protocol

Sample Preparation:

1. Serum and Plasma samples can be measured directly.
2. Urine Samples need to be spun down at 10,000 x g for 5 min at RT to collect the supernatant.
3. Tissue (~10 mg) or cells (~1 x 107) should be rapidly homogenized with 100 µl ice cold Assay Buffer XXXII/D2HG Assay Buffer for 10 min on ice. Centrifuge at 10,000 x g, 4°C for 5 min, collect the supernatant.
4. Add the same volume (0-45 µl) of each Sample into three wells of a 96 well clear plate.

Δ 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 with the added benefit of removal of possible interfering enzyme activity to remove the insoluble components. Use the flow through for the assay.

Standard Preparation:

1. 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 Assay Buffer XXXII/D2HG Assay Buffer and mix well.

Internal Standard:

1. 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.
2. 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 Assay Buffer XXXII/D2HG Assay Buffer.

Reaction Mix Preparation:

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

Item	Reaction Mix	*Background Control Mix
Assay Buffer XXXII/D2HG Assay Buffer	46 µl	48 µl
D2HG Enzyme	2 µl	0 µl
Developer Solution III/D2HG Substrate Mix	2 µl	2 µl

2. Add 50 µl of the Reaction Mix to each well containing the Standards and Samples. Mix well.

Δ 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.

Measurement

Incubate the plate for 60 min at 37°C and measure OD450 nm.

Calculation

1. 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:

$$\textbf{D2HG amount (nmol)} = \left(\frac{(OD\ sample\ (corrected))}{(OD\ (spiked\ sample))-(OD\ sample)}\right) \textbf{ x 5}$$

2. The D2HG concentration in the Sample is:

$$\textbf{C = X/V x D = nmol/µl = mmol/l or mM}$$

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)

Δ Notes:

a) D2HG MW = 192.08.

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

Technical Support

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