

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.

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 32	20 mL	-20°C
D2HG Enzyme	1 vial	-20°C
Developer Solution III	1 vial	-20°C
D2HG Standard	1 vial	-20°C

PLEASE NOTE: Assay Buffer 32 was previously labelled as Assay Buffer XXXII and D2HG Assay Buffer, and Developer Solution III as D2HG Substrate Mix. The composition has not changed.

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 32: Allow the Assay Buffer 32 to warm to room temperature (RT) prior to use.

D2HG Enzyme: Reconstitute with 220 µl Assay Buffer 32. 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: 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.

Δ Note: If samples are not clear, they need to be clarified through a 0.22 µm filter or spin filtered using a 10kD Spin column (ab93349) in order to remove insoluble components. Use flow through for the assay.

Δ Note: If background control produces OD values greater than sample OD values, the samples may contain endogenous interfering enzyme activity. These enzymes can be removed from the sample by filtering through a 10kD Spin column (ab93349).

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 32 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 32 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 32.

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 32	46 µl	48 µl
D2HG Enzyme	2 µl	0 µl
Developer Solution III	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:

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

2. The D2HG concentration in the Sample is:

$$C = X/V \times D = \text{nmol}/\mu\text{l} = \text{mmol}/\text{l or mM}$$

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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