ab284542 – Human GAPDH Activity Assay Kit II

For the measurement of GAPDH activity in various tissues and cells and the analysis of glycolysis and pentose phosphate pathways
For research use only – not intended for diagnostic use

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284542

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
GAPDH Assay Buffer	25 mL	-20°C
GAPDH Substrate	200 µL	-20°C
GAPDH Developer	1 Vial	-20°C
NADH Standard	1 Vial	-20°C
Human GAPDH Positive Control	1 Vial	-20°C
GAPDH Reconstitution Buffer	1.5 mL	-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 clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

Reagent Preparation

GAPDH Assay Buffer & GAPDH Reconstitution Buffer: Warm to room temperature (RT) before use. Store at -20°C.

GAPDH Substrate: Reconstitute the vial with 220 µL of GAPDH Assay Buffer. Pipette up and down to dissolve completely. Keep on ice while in use. Divide into aliquots and store at -20°C. Use within two months.

GAPDH Developer: Reconstitute the vial with 220 µL ddH2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.

NADH Standard: Reconstitute the vial with 400 µL ddH2O to generate 1.25 mM (1.25 nmol/µl) NADH stock Standard solution. Keep on ice while in use. Divide into aliquots and store at -20°C. Use within two months.

Human GAPDH Positive Control: Reconstitute the vial with 100 µL GAPDH Reconstitution Buffer. Vortex several times and put on ice for 5 min to completely dissolve. Divide into aliquots and store at -20°C. Avoid freeze/thaw cycles. Keep on ice while in use. Use within two months.

Assay Protocol

Sample preparation:

1. For whole cells or tissue lysate, rapidly homogenize tissue (~10 mg) or cells (1 x 106) with 100 µL GAPDH Assay Buffer and keep on ice for 10 min.
2. Centrifuge at 10,000 x g for 5 min and collect the supernatant. Dilute the cell lysates 50-fold by adding 10 µL of cell lysates to 490 µL of GAPDH Assay Buffer and mix well.
3. Add 1-50 µL of diluted sample(s) per well. Adjust the volume to 50 µL/well with GAPDH Assay Buffer.
4. For GAPDH Positive Control, dilute the reconstituted Human GAPDH Positive Control 10-fold by adding 20 µL of the reconstituted Human GAPDH to 180 µL of GAPDH Reconstitution Buffer.
5. Add 1-20 µL of diluted Human GAPDH Positive Control into respective wells and adjust the volume to 50 µL/well with GAPDH Assay Buffer.

Δ Note:

a) For Unknown Samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

b) For samples having background, prepare a parallel sample well labeled as Sample Background Control.

Standard Curve Generation:

1. Add 0, 2, 4, 6, 8 and 10 µL of 1.25 mM NADH stock Standard into a series of wells in 96 well clear plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well of NADH Standard.
2. Adjust the volume to 50 µL/well with GAPDH Asset Buffer.

Reaction Mix Preparation:

1. Mix enough reagents for the number of assays to be performed. For each well, prepare a total of 50 µl:

Item	Reaction Mix	Background Control Mix*
GAPDH Assay Buffer	46 µL	48 µL
GAPDH Substrate	2 µL	-
GAPDH Developer	2 µL	2µL

2. Add 50 µL of Reaction Mix to each well containing Standard, Positive Control and sample(s).
3. *For samples having high background, add 50 µL of Background Control Mix to the Sample Background Control well. Mix well.

Measurement

Measure the plate at 450 nm in kinetic mode for 10-60 min. at 37°C.
Δ Note: Incubation time depends on the GAPDH activity in the samples. We recommend measuring the OD in a kinetic mode and choosing any two time points (T1 & T2) in the linear range to calculate the GAPDH activity of the samples. The NADH Standard Curve can be read in End point mode (i.e. at the end of sample incubation time).

Calculation

1. Subtract the 0 Standard reading from all Standard readings and Sample Background Control reading from all sample readings respectively.
2. Plot the NADH Standard Curve.
3. Choose any two time points within the linear portion of the curve (T1 & T2) for each sample type.
4. Subtract the Sample Background Control readings from the corresponding sample readings for the chosen T1 & T2 time points.
5. Apply the corrected sample readings to the NADH Standard Curve to get B nmol of NADH generated during the reaction time (ΔT = T2 - T1).

   Sample GAPDH Activity = B/(ΔT X V) x D = nmol/min/µL = mU/µL = U/mL

   Where:
   B = NADH amount from the Standard Curve (nmol)
   ΔT = Reaction time (min)
   V = Sample volume used (µL)
   D = Sample dilution factor
   Unit Definition: One unit of GAPDH is the amount of enzyme that will generate 1.0 µmol of NADH per min. at pH 8.0 at 37°C.

Technical Support

For all technical or commercial enquiries please go to:
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