ab204732
Glyceraldehyde 3 Phosphate Dehydrogenase Activity Assay Kit (Colorimetric)

Instructions for Use

For rapid, sensitive and accurate detection of Glyceraldehyde 3 Phosphate Dehydrogenase Activity.

This product is for research use only and is not intended for diagnostic use.

Version 4a, Last Updated 2 July 2025

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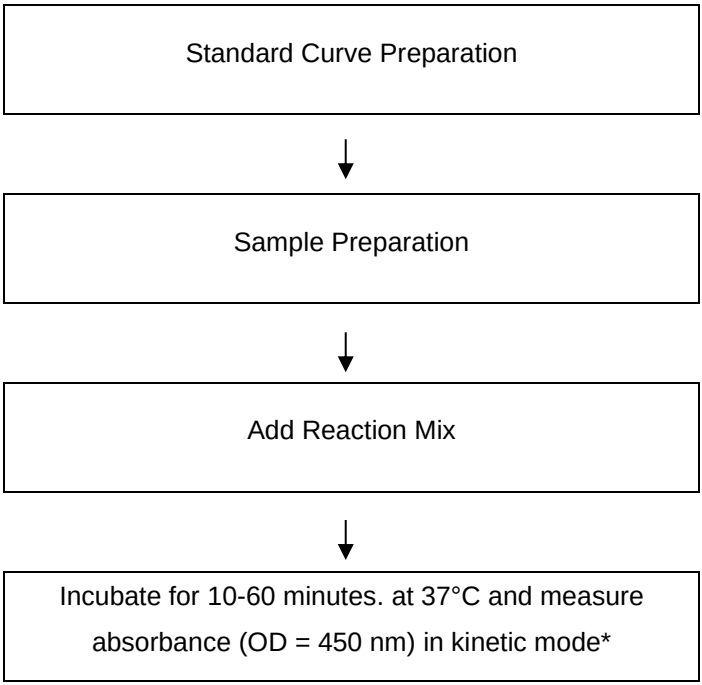
1. BACKGROUND

Glyceraldehyde 3 Phosphate Dehydrogenase Activity Assay Kit (Colorimetric) (ab204732) provides a simple and sensitive method for monitoring Glyceraldehyde 3 Phosphate Dehydrogenase (GAPDH) activity in various samples. In this assay, GAPDH catalyzes conversion of GAP into BPG and an intermediate, which reacts with a developer to form a colored product that absorbs maximally at 450 nm. Our high-throughput adaptable assay can detect GAPDH activity as low as 100 µU/mL in a variety of samples.

GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase; EC 1.2.1.12) catalyzes the conversion of Glyceraldehyde-3-Phosphate (GAP) to 1, 3-Bisphosphate Glycerate (BPG) and plays a key role in glycolysis. The enzyme is involved in cellular processes such as apoptosis, membrane trafficking, iron metabolism and nuclear translocation. GAPDH (housekeeping gene) expression is stable and constitutive. Deregulation of GAPDH activity is associated with abnormal cell proliferation and carcinogenesis. Accurate quantitation of GAPDH activity is important for diagnosing diseases and studying normal cellular physiology.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 4	25 mL	-20°C	-20°C
Glyceraldehyde-3-Phosphate	200 µL	-20°C	-20°C
Developer Solution III	1 Vial	-20°C	-20°C
NADH Standard I	1 Vial	-20°C	-20°C
GAPDH Positive Control	1 Vial	-20°C	-80°C

PLEASE NOTE: Assay Buffer 4 was previously labelled as Assay Buffer IV and GAPDH Assay Buffer. The composition has not changed.

7. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips
- Microcentrifuge
- Multi-well spectrophotometer
- 96 well plate with clear flat bottom
- Heat block or water bath
- Dounce homogenizer

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 4:**

Ready to use as supplied. Equilibrate Assay Buffer to room temperature before use. Store at -20°C.

9.2 **Glyceraldehyde-3-Phosphate:**

Ready to use as supplied. Keep on ice while in use. Divide into aliquots and store at -20°C. Use within two months.

9.3 **Developer Solution III:**

Reconstitute the Developer Solution III with 220 µL ddH_2O. Pipette up and down to dissolve completely. Aliquot developer so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months.

9.4 **NADH Standard I:**

Reconstitute the NADH Standard I with 400 µL ddH_2O to generate a 1.25 mM (1.25 nmol/µL) NADH Standard I solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months. Keep on ice while in use.

9.5 **GAPDH Positive Control:**

Reconstitute the GAPDH Positive Control with 100 µL ddH_2O and mix thoroughly. Aliquot Positive Control so that you have enough volume to perform the desired number of assays. Store at -80°C after preparation. Use within two months. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and cannot be stored for future use.

10.1 Using 1.25 mM NADH Standard I, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Conc NADH in well (nmol/well)
1	0	150	50	0
2	6	144	50	2.5
3	12	138	50	5.0
4	18	132	50	7.5
5	24	126	50	10.0
6	30	120	50	12.5

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of ice cold Assay Buffer 4.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Incubate cells on ice for 10 minutes.

11.1.6 Centrifuge sample at 10,000 x *g* for 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.1.7 Collect supernatant and transfer to a clean tube.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of ice cold Assay Buffer 4. Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.4 Incubate sample on ice for 10 minutes.

11.2.5 Centrifuge sample at 10,000 x *g* for 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **Prepare all reagents, working standards, and samples as directed in the previous sections.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 4).
- Background control sample wells= 1 - 50 µL samples (adjust volume to 50 µL/well Assay Buffer 4).
- Positive control = 2 - 20 µL GAPDH Positive control (adjust volume to 50 µL/well with Assay Buffer 4).

12.2 **Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer 4	**46**	**48**
Developer Solution III	**2**	**2**
Glyceraldehyde-3-Phosphate	**2**	**-**

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1).

12.3 Add 50 µL of Reaction Mix into each standard, sample and positive control sample wells. Mix well.

12.4 Add 50 µL of Background Reaction Mix to Background control sample wells.

12.5 Mix well.

12.6 Measure output at OD450 nm on a microplate reader in a kinetic mode, every 2 – 3 minutes, for at least 10 minutes at 37°C protected from light.

NOTE: *Incubation time depends on the GAPDH Activity in the samples. We recommend measuring OD in a kinetic mode, and choosing two time points (T_1 and T_2) in the linear range (OD values A_1 and A_2 respectively) to calculate the GAPDH activity of the samples. The Standard Curve can be read in end point mode (i.e. at the end of incubation time).*

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of inosine.

13.5 Draw the best smooth curve through these points to construct the standard curve. Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Amount of NADH is calculated as:

$$\mathbf{\Delta A_{450nm} = (A_2 - A_{2BG}) - (A_1 - A_{1BG})}$$

Where:

A1 is the sample reading at time T1.

A_{1BG} is the background control sample at time T1.

A2 is the sample reading at time T2.

A_{2BG} is the background control sample at time T2.

13.7 Use the ΔA_{450nm} to obtain B nmol of NADH generated by GAPDH during the reaction time ($\Delta T = T_2 - T_1$).

13.8 Concentration of GAPDH in the test samples is calculated as:

$$GAPDH\ Activity = \left(\frac{B}{\Delta T x\ V}\right) * D = nmol/min/\mu L = mU/\mu L$$

$$= U/mL$$

Where:

B = Amount of NADH from Standard Curve (nmol).

ΔT = Reaction time (minutes).

V = Original sample volume added into the reaction well (µL).

D = Sample dilution factor.

GAPDH activity can also be expressed as U/mg of total protein in the sample.

Unit Definition:

1 Unit GAPDH activity = amount of Glyceraldehyde 3 Phosphate Dehydrogenase that will generate 1.0 µmol of NADH per minute at pH 7.2 at 37°C.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

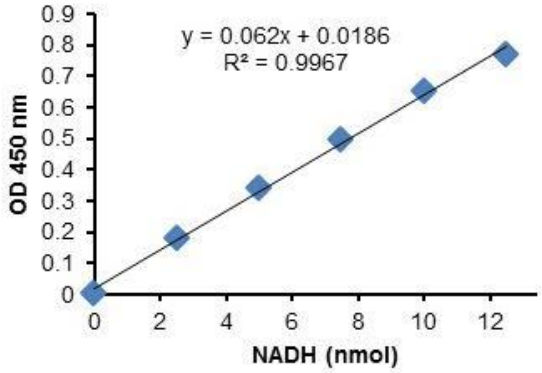


Figure 1. Typical NADH Standard calibration curve.

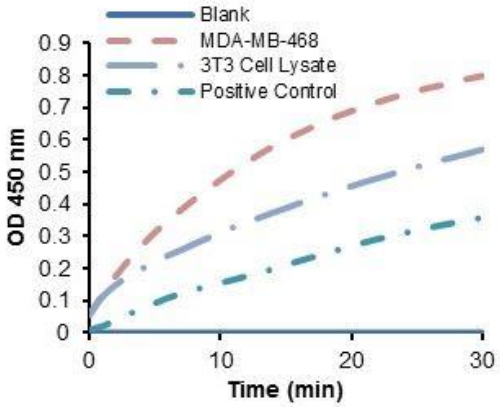


Figure 2. GAPDH activity in the Positive Control, 3T3 cells lysate and MDA-MB-468 cells lysate.

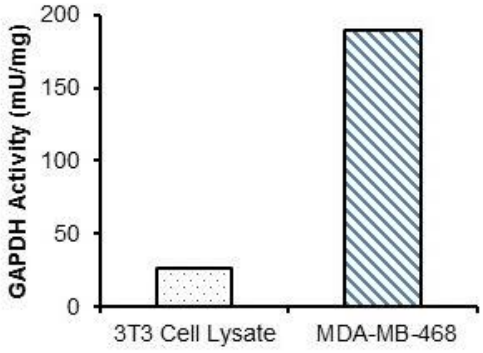


Figure 3. GAPDH specific activity calculated from 3T3 cell lysate (11.1 µg protein), and MDA-MB-468 cell lysate (2.76 µg protein).

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, positive control and prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL), positive control (50 µL) and background wells (50 µL).
- Prepare GAPDH Reaction Mix (Number samples + standards + 1).

Component	Reaction Mix (µL)	Bckg Reaction Mix (µL)
Assay Buffer 4	**46**	**48**
Developer Solution III	**2**	**2**
Glyceraldehyde-3-Phosphate	**2**	**-**

- Add 50 µL of GAPDH Reaction Mix to the standard and sample wells.
- Add 50 µL of Background Reaction Mix into the background sample control wells. Mix well.
- Incubate plate at 37°C for 10 - 60 minutes and read absorbance at OD= 450 nm.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Clear plates
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17.INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- RIPA buffer – it contains SDS which can destroy/decrease the activity of the enzyme.

18.**NOTES**

Technical Notes:

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)