

# GAPDH Activity Assay Kit

PromoKine

Simple, rapid and sensitive colorimetric measurement of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity in various tissues/cells

## Instruction Manual

Catalog Number	PK-CA577-K680																		
Description	<p>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.</p> <p>PromoCell's <i>GAPDH Activity Assay Kit</i> provides a simple and sensitive method for monitoring GAPDH activity in various samples. In this assay, GAPDH catalyzes the 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 <math>\mu</math>U/ml in a variety of samples.</p> <p style="text-align: center;"><math>\text{GAP} + \text{Phosphate} + \text{NAD}^+ \xrightarrow{\text{GAPDH}} \text{BPG} + \text{Intermediate} \xrightarrow{\text{Developer}} \text{Color detection (OD450 nm)}</math></p>																		
Applications	<ul style="list-style-type: none"><li>• Measurement of GAPDH activity in various tissues and cells</li><li>• Analysis of glycolysis and pentose phosphate pathway</li></ul>																		
Sample Type	<ul style="list-style-type: none"><li>• Animal tissues: Liver, heart etc.</li><li>• Cell culture: Adherent or suspension cells</li></ul>																		
Quantity	100 assays																		
Kit Components	<table border="1"><thead><tr><th>Components</th><th>Quantity</th><th>Color Code</th></tr></thead><tbody><tr><td>GAPDH Assay Buffer</td><td>25 ml</td><td>WM</td></tr><tr><td>GAPDH Substrate</td><td>200 <math>\mu</math>l</td><td>Blue</td></tr><tr><td>GAPDH Developer (lyophilized)</td><td>1 vial</td><td>Red</td></tr><tr><td>NADH Standard (500 nmol, lyophilized)</td><td>1 vial</td><td>Yellow</td></tr><tr><td>GAPDH Positive Control (lyophilized)</td><td>1 vial</td><td>Orange</td></tr></tbody></table>	Components	Quantity	Color Code	GAPDH Assay Buffer	25 ml	WM	GAPDH Substrate	200 $\mu$ l	Blue	GAPDH Developer (lyophilized)	1 vial	Red	NADH Standard (500 nmol, lyophilized)	1 vial	Yellow	GAPDH Positive Control (lyophilized)	1 vial	Orange
Components	Quantity	Color Code																	
GAPDH Assay Buffer	25 ml	WM																	
GAPDH Substrate	200 $\mu$ l	Blue																	
GAPDH Developer (lyophilized)	1 vial	Red																	
NADH Standard (500 nmol, lyophilized)	1 vial	Yellow																	
GAPDH Positive Control (lyophilized)	1 vial	Orange																	
User Supplied Reagents & Equipment	<ul style="list-style-type: none"><li>• Microplate reader capable of absorbance measurement</li><li>• 96-well clear plate with flat bottom</li></ul>																		
Storage and Reagents Preparation	<p>Store kit at <math>-20^{\circ}\text{C}</math>, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.</p> <ul style="list-style-type: none"><li>• <b>GAPDH Assay Buffer:</b> Warm to room temperature (RT) before use. Store at <math>-20^{\circ}\text{C}</math>.</li><li>• <b>GAPDH Substrate:</b> Ready to use as supplied. Keep on ice while in use. Divide into aliquots and store at <math>-20^{\circ}\text{C}</math>. Use within two months.</li><li>• <b>GAPDH Developer:</b> Reconstitute with 220 <math>\mu</math>l ddH<sub>2</sub>O. Pipette up and down to dissolve completely. Store at <math>-20^{\circ}\text{C}</math>. Use within two months.</li><li>• <b>NADH Standard:</b> Reconstitute with 400 <math>\mu</math>l ddH<sub>2</sub>O to prepare a 1.25 mM (1.25 nmol/<math>\mu</math>l) NADH Standard solution. Keep on ice while in use. Aliquot and store at <math>-20^{\circ}\text{C}</math>. Use within two months.</li><li>• <b>GAPDH Positive Control:</b> Reconstitute with 100 <math>\mu</math>l ddH<sub>2</sub>O and mix thoroughly. Keep on ice while in use. Aliquot and store at <math>-70^{\circ}\text{C}</math>. Use within two months.</li></ul>																		
Assay Protocol	<p><b>1. Sample Preparation:</b> For whole cells (<math>1 \times 10^6</math>) or tissues (~10 mg), rapidly homogenize with 100 <math>\mu</math>l GAPDH Assay Buffer, and keep on ice for 10 minutes. Centrifuge at 10,000 x g, <math>4^{\circ}\text{C}</math> for 5 minutes and collect the supernatant for the assay. Add 1-50 <math>\mu</math>l sample per well, adjust final volume to 50 <math>\mu</math>l with GAPDH Assay Buffer. Add 2-20 <math>\mu</math>l of GAPDH Positive Control into wells and adjust final volume to 50 <math>\mu</math>l with GAPDH Assay Buffer. Notes:</p> <p>a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve linear range.</p>																		

b. For samples having background, prepare parallel sample wells for sample background controls.

**2. NADH Standard Curve:** Add 0, 2, 4, 6, 8 and 10  $\mu\text{l}$  of 1.25 mM NADH Standard into a series of wells in 96 well plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well of NADH Standard. Adjust volume to 50  $\mu\text{l}$ /well with GAPDH Assay Buffer.

**3. Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50  $\mu\text{l}$  *Reaction Mix* containing:

	Reaction Mix	Background Control Mix*
GAPDH Assay Buffer	46 $\mu\text{l}$	48 $\mu\text{l}$
GAPDH Developer	2 $\mu\text{l}$	2 $\mu\text{l}$
GAPDH Substrate	2 $\mu\text{l}$	----

Add 50  $\mu\text{l}$  of the Reaction Mix to each well containing the Standards, Positive Control and test samples.

\*For samples having high background, add 50  $\mu\text{l}$  of Background Control Mix to each well and mix well.

**4. Measurement:** Measure the plate at 450 nm in kinetic mode for 10-60 minutes at 37°C.

Note: Incubation time depends on the GAPDH activity in the samples. We recommend measuring the OD in a kinetic mode and choosing 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).

**5. Calculation:** Subtract the 0 Standard OD value from all Standard readings. Plot the NADH Standard Curve. Subtract the background control OD value from all sample readings. Calculate the  $\Delta\text{OD} = \text{A2} - \text{A1}$  for the GAPDH activity of the test sample. Apply the  $\Delta\text{OD}$  to the NADH standard curve to get B nmol of NADH generated by GAPDH activity during the reaction time ( $\Delta\text{T} = \text{T2} - \text{T1}$ ).

$$\text{Sample GAPDH Activity} = \text{B}/(\Delta\text{T} \times \text{V}) \times \text{D} [= \text{nmol}/\text{min}/\mu\text{l} = \text{mU}/\mu\text{l} = \text{U}/\text{ml}]$$

Where: B is the NADH amount from standard curve (nmol)

$\Delta\text{T}$  is the reaction time (min.)

V is the sample volume added into the reaction well ( $\mu\text{l}$ )

D is dilution factor

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

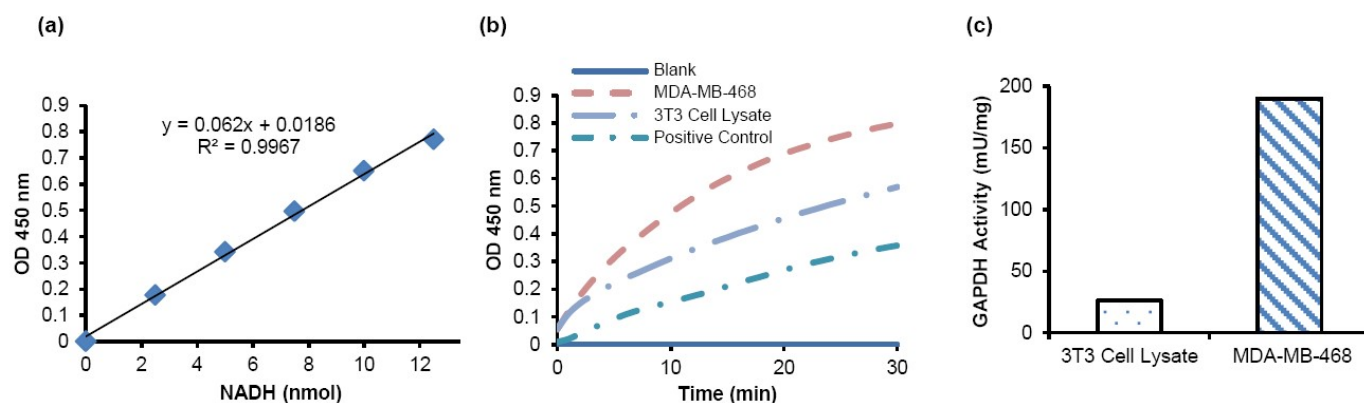
Unit Definition: One unit of GAPDH is the amount of enzyme that will generate 1.0  $\mu\text{mol}$  of NADH per minute at pH 7.2 at 37°C.

#### Related Products

- GAPDH Inhibitor Screening Kit (Cat.No. PK-CA577-K2043)
- GAPDH Antibodies & Blocking Peptides (Cat.Nos. PK-AB718-3781 / -3781P, PK-AB718-3783 / P3783P, PK-AB718-5003, PK-AB718-5007)
- Find more products for studying the Glycolysis and related pathways at [www.promocell.com](http://www.promocell.com).

#### Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.



**Figure:** (a). NADH Standard Curve. (b). GAPDH activity in the Positive Control, 3T3 cells lysate and MDA-MB-468 cells lysate. (c). GAPDH specific activity calculated from 3T3 cell lysate (11.1  $\mu\text{g}$  protein), and MDA-MB-468 cell lysate (2.76  $\mu\text{g}$  protein). Assays were performed following kit protocol.

#### PromoCell GmbH

Sickingenstr. 63/65  
69126 Heidelberg  
Germany

Email: [info@promocell.com](mailto:info@promocell.com)  
[www.promocell.com](http://www.promocell.com)

#### USA/Canada

Phone: 1 – 866 – 251 – 2860 (toll free)  
Fax: 1 – 866 – 827 – 9219 (toll free)

#### Deutschland

Telefon: 0800 – 776 66 23 (gebührenfrei)  
Fax: 0800 – 100 83 06 (gebührenfrei)

#### France

Téléphone: 0800 90 93 32 (ligne verte)  
Téléfax: 0800 90 27 36 (ligne verte)

#### United Kingdom

Phone: 0800 – 96 03 33 (toll free)  
Fax: 0800 – 169 85 54 (toll free)

#### Other Countries

Phone: +49 6221 – 649 34 0  
Fax: +49 6221 – 649 34 40