Rev. 2

Colorimetric GAPDH Assay (GAPDH)

Catalog #8148
100 tests

Product Description

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) is a tetrameric enzyme that catalyzes glycolysis and thus serves to break down glucose for energy and carbon molecules. Since it is ubiquitously expressed at relatively constant levels, GAPDH gene is widely used as an internal control for RNA and protein analysis. The ScienCell™ Colorimetric GAPDH Assay provides a non-radioactive method to measure the enzyme activity of GAPDH in cultured cells, based on the oxidization of β-NADH to β-NAD in the presence of 3-phosphoglyceric acid (3-PGA), adenosine 5'-triphosphate (ATP) and GAPDH. The GAPDH activity is determined by assaying the rate of NADH oxidation, which is proportional to the reduction in absorbance at 340 nm over time (ΔA_{340nm}/min).

Kit Components

Cat. No.	# of vials	Name	Quantity	Storage
8148a	1	Cell Lysis Buffer	10 mL	4ºC
8148b	1	GAPDH Assay Buffer	12.5 mL	4ºC
8148c	1	3-PGA	1 mL	-20ºC
8148d	2	L-Cysteine	1 mL	-20ºC
8148e	1	β-NADH	0.25 mL	-20ºC
8148f	1	ATP	0.5 mL	-20ºC
8148g	1	3-PGK	0.125 mL	-20ºC
8148h	1	GAPDH Positive Control	20 µL	-20ºC

Materials supplied by user:

Microplate reader
Phosphate-Buffered Saline (PBS)
96-well plate

Quality Control

Diluted GAPDH positive control is measured with the GAPDH Assay kit after different time of reaction. The detection limit is 0.0625 to 2 units/mL. The GAPDH Assay is also applied to the lysate of primary cells cultured in 24-well plate for 3 hours, with serially diluted seeding densities (5-1.25×10^4 cells per well). The resulting ΔA_{340nm} at 3-12 minutes of reaction, and the ΔA_{340nm}/min vs. cell seeding density curve are shown in Figure 3.

Product Use

This assay kit is used to evaluate GAPDH activity *in vitro*. This kit is for research use only and is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping

Dry Ice.

Procedure

A. Preparation of GAPDH Positive Control

1. Prepare the GAPDH positive control by diluting it 5X. For example, mix 2.5 µL of the enzyme with 10 µL of PBS. Then, add 5 µL of the diluted GAPDH positive control into two wells of a 96-well flat-bottom plate.

B. Preparation of test samples and blank

1. Remove culture medium from the cultured cells, wash cells twice with ice-cold PBS and remove PBS.
2. Add 100 µl of ice-cold Cell Lysis Buffer to each sample well of 24-well plate (~0.1-1×10^5 cells) and gently rock the plate side-to-side. For cells in different size wells, scale up or down the volume of Cell Lysis Buffer according to the surface area of the wells.
3. Incubate at 2-8ºC for 20 minutes with gentle agitation to lyse cells. Centrifuge the lysate at 14,000 × g in pre-cooled centrifuge for 3 minutes, transfer the supernatant to fresh tube and discard the pellet. Cell lysate can be stored at -70 ºC or used immediately for GAPDH measurement.
4. Samples should be serial diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 5 µL/well on the 96-well flat bottom plate.
5. Prepare blanks by adding 5 µL assay buffer (8148b) into two wells on the 96-well flat bottom plate.

C. GAPDH Assay procedure

1. Prepare appropriate volume of GAPDH assay mixture based on the number of samples to be measured. For each sample, prepare 145 µL of GAPDH assay mixture according to Table 1.

Table 1. Preparation of GAPDH assay mixture (145µL per well).

Reagent	Volume (µL)
GAPDH Assay Buffer	106.25
3-PGA	10
L-Cysteine	20
β-NADH	2.5
ATP	5
3-PGK	1.25
Total	145

2. Add 145 µL of working reagent mix into each well of the 96-well plate containing diluted GAPDH positive control, samples and blank, mix well immediately and start recording A_{340nm} over a 12 minutes interval, collecting data every 1 minute. Figure 1 shows data of GAPDH positive control.

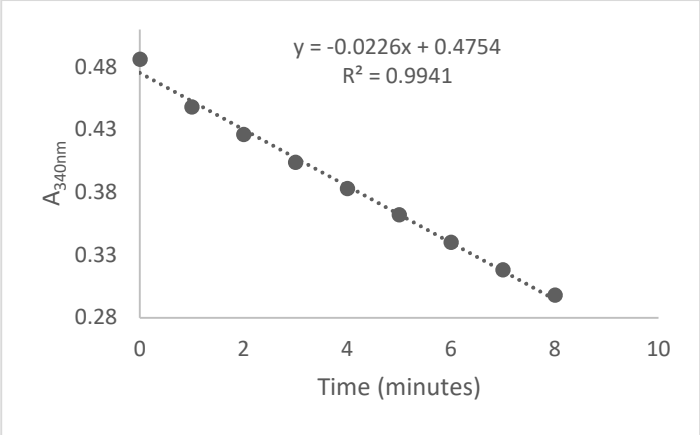

Figure 1. Absorbance changes of diluted GAPDH positive control at 340nm.

*The interval of measurement depends on the concentration of GAPDH. For GAPDH solutions of 0.2-1 units/mL, an interval of 3-12 minutes ensures a linear relationship between ΔA_{340nm}/min and GAPDH concentration. Generally, longer reaction time is needed for lower concentration of GAPDH, and vice versa.

D. Calculations:

1. Determine the change in absorbance ΔA_{340}nm/min by plotting the absorbance value at ΔA_{340nm} as a function of reaction time to obtain the slope of the linear portion of the curve. As shown in Figure 2.

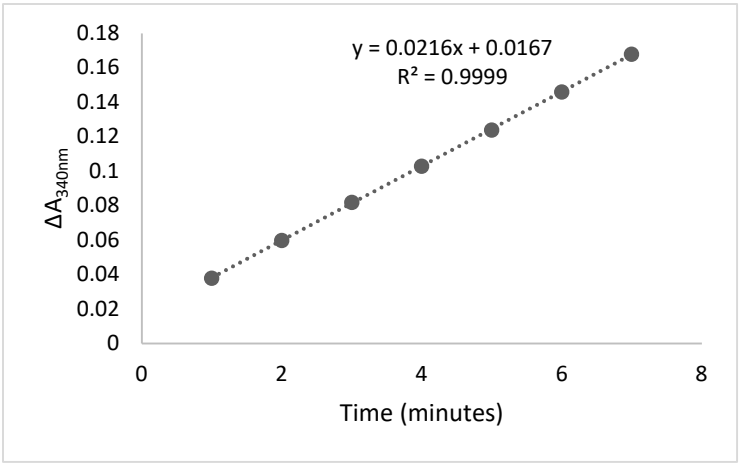

Figure 2. The change in absorbance ΔA_{340nm} of diluted GAPDH positive control during the time at 340nm

2. Calculate the GAPDH activity based on the following formula:

$$GAPDH\ U/mL = \frac{\Delta A_{340nm}/\text{min} \times \text{V total}}{1.94\ \text{mM}^{-1}\text{cm}^{-1} \times \text{V sample}} \times sample\ dilution$$

Note: The actual extinction coefficient for NADH at 340 nm is 6.22 $mM^{-1}cm^{-1}$, which has been adjusted to 1.94 $mM^{-1}cm^{-1}$ to account for the reduced path length in the 96-well plate.

ΔA_{340nm}/min = Change in absorbance per unit time (slope)

V total = 150 µL = 0.150 mL

Adjusted extinction coefficient for NADH at 340 nm in the 96-well plate = **1.94 $mM^{-1}cm^{-1}$**

V sample = 5 µL = 0.005 mL

Unit definition: One unit would make 1.0 µmol of NADH to NAD^+ per minute at pH 7.4 at 25 ºC.

3. Use the formula to calculate GAPDH positive control activity:

$$GAPDH(U/mL) = \Delta A340nm/min \times \frac{0.150}{1.94 \times 0.005} \times sample\ dilution$$

$$GAPDH(U/mL) = \Delta A340nm/min \times 15.46 \times sample\ dilution$$

GAPDH Positive control (U/mL) = 0.021×15.46 × 5= 1.6 U/mL

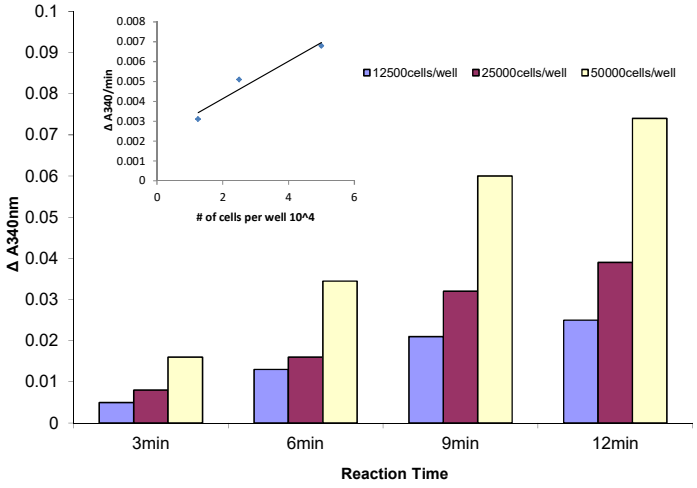

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Figure 3. Human Dermal Fibroblasts (HDFs) are seeded at serially diluted densities (5-1.25×10^4 cells per well) in a 24-well plate and cultured for 3 hours. The ScienCell™ Colorimetric GAPDH Assay is then applied to the corresponding cell lysate collected. The ΔA_{340nm} of each lysate measured during a time interval of 3-12 minutes and the resulting ΔA_{340nm}/min vs. cell seeding density curve are shown. A positive linear relationship can be observed between the GAPDH activity, which is represented by the ΔA_{340nm}/min, and the number of adherent cells, which is proportional to the cell seeding density.