Colorimetric GAPDH Assay
Cat. No. 8148, 100 tests

Introduction

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	100 mM 3-PGA	1 ml	-20ºC
8148d	1	100 mM L-Cysteine	0.5 ml	-20ºC
8148e	1	7 mM β-NADH	0.25 ml	-20ºC
8148f	1	34 mM ATP	0.5 ml	-20ºC
8148g	1	400 units/ml 3-PGK	0.125 ml	-20ºC
8148h	1	GAPDH standard (200 units/ml)	20 µl	-20ºC

Product Use

This assay kit is used to evaluate GAPDH activity *in vitro*. It is for research use only. Not for use in animals, humans, or diagnostic procedures.

Quality Control

Serially diluted GAPDH solutions with concentrations ranging from 0.2 to 1 units/ml are measured with the ScienCell™ Colorimetric GAPDH Assay after different time of reaction, and the resulting standard curves are shown in Figures 1-3. Positive linear relationship between ΔA_{340nm} & GAPDH concentration at different reaction time (Figure 1), ΔA_{340nm} & reaction time with different GAPDH concentrations (Figure 2), and ΔA_{340nm}/min & GAPDH concentration (Figure 3) can be observed.

The ScienCell™ Colorimetric GAPDH Assay is also applied to the lysate of Human Dermal Fibroblasts (HDFs) 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 4, indicating a positive relationship between GAPDH activity and seeding density.

Procedures

A. Preparation of GAPDH standards

1. Dilute 200 units/ml GAPDH standard to 1unit/ml using PBS. Prepare a GAPDH standard curve using the serial dilutions of the 1 unit/ml GAPDH standard according to Table 1. Fifteen µl of

GAPDH solution is prepared to provide three replicates of 5 µl for each point of the standard curve.

B. Preparation of cell lysate

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 min 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.

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 2, add to 96-well plate. Measure the initial A_{340nm} of the GAPDH assay mixtures.
2. Transfer 5 µl of each sample or standard to each well the 96-well plate containing 145 µl of GAPDH assay mixture, mix well immediately and start recording A_{340nm} over an 12 minute interval, collecting data every 3 min.*

D. Calculations:

1. Subtract the measured A_{340nm} at different reaction time from the initial A_{340nm} to obtain the corresponding ΔA_{340nm} for each sample and GAPDH standard at different reaction time. Average the ΔA_{340nm} of replicate wells.
2. Based on the ΔA_{340nm} of the GAPDH standard solutions, two kinds of standard curves can be made: First, the standard curve of ΔA_{340nm} vs. GAPDH concentration at reaction time (e.g. Figure 1). Second, the standard curve of ΔA_{340nm}/min vs. GAPDH concentration (e.g. Figure 3), in which the ΔA_{340nm}/min is calculated by plotting the ΔA_{340nm} as a function of reaction time as shown in Figure 2.
3. Calculate the GAPDH concentration of the samples based on the standard curve.

*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.

No.	1 unit/ml GAPDH (µL)	PBS (µL)	GAPDH concentration (units/ml)
1	15	0	1
2	12	3	0.8
3	9	6	0.6
4	6	9	0.4
5	3	12	0.2
6	0	15	Blank

Table 1. Preparation of GAPDH standards.

Reagent	Volume (µl)
GAPDH Assay Buffer	121.25
100 mM 3-PGA	10
100 mM L-Cysteine	5
7 mM β-NADH	2.5
34 mM ATP	5
400 Units/ml 3-PGK	1.25
Total	145

Table 2. Preparation of GAPDH assay mixture (145µl per sample).

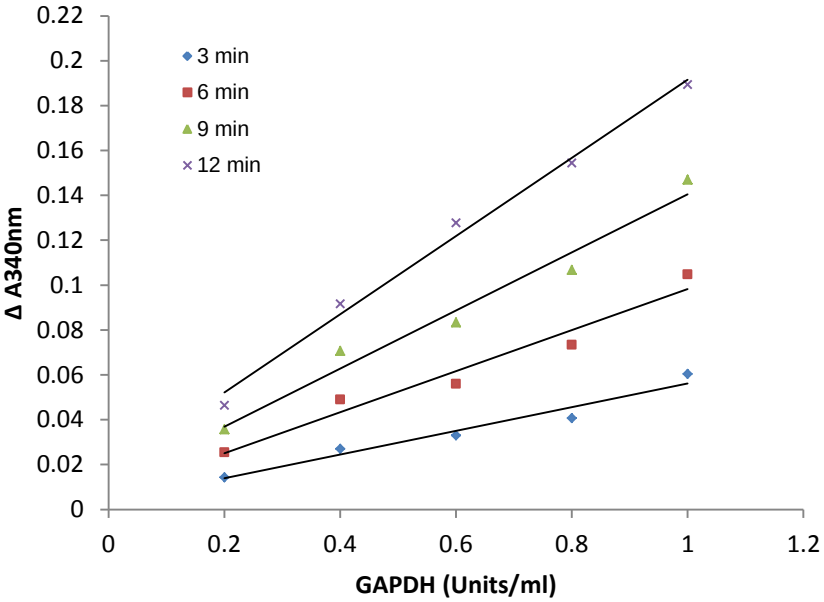

Figure 1. Standard curves of ΔA_{340nm} vs. GAPDH concentration measured after 3, 6, 9, and 12 minutes of reaction.

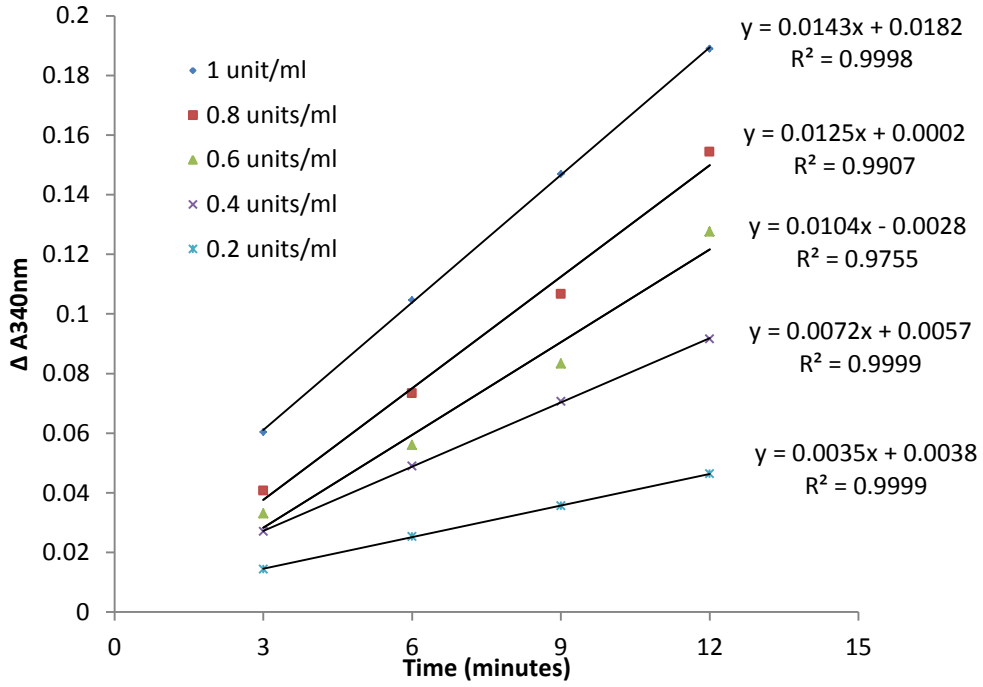

Figure 2. Standard curves of ΔA_{340nm} vs. reaction time for GAPDH solution with different concentrations.

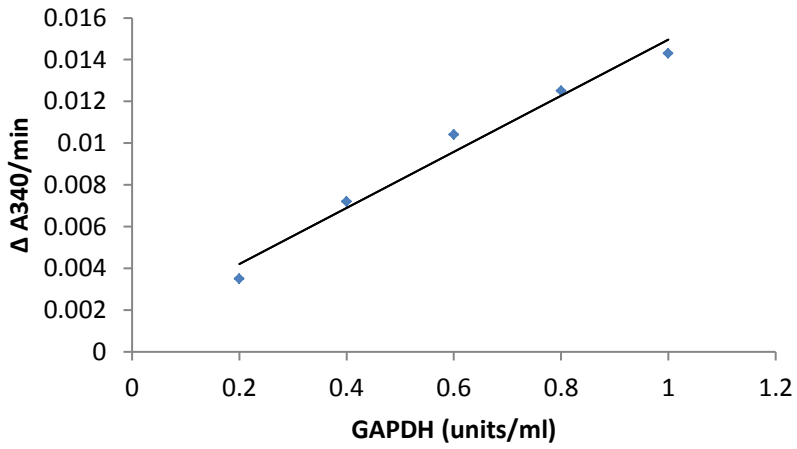

Figure 3. A standard curve of ΔA_{340nm}/min vs. GAPDH concentration, wherein the ΔA_{340nm}/min is calculated as the slope of the standard curves shown in Figure 2.

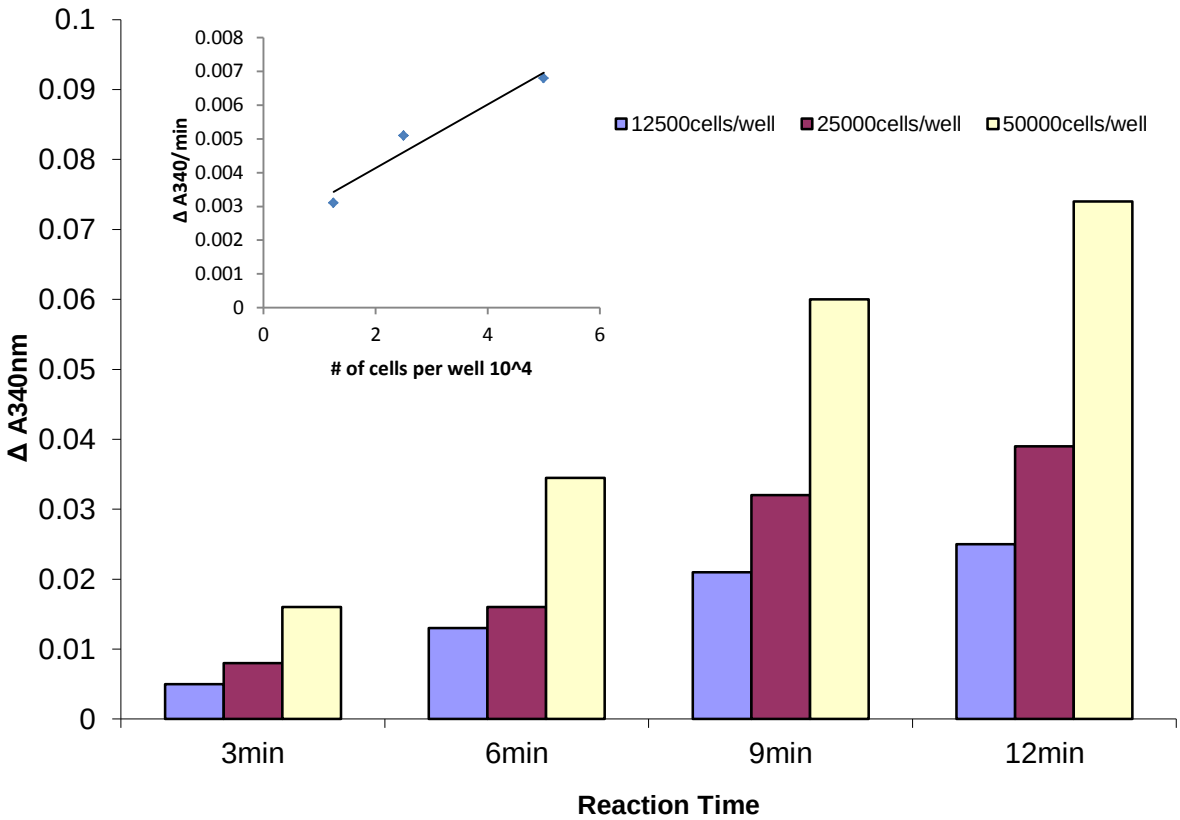

Figure 4. Human Dermal Fibroblasts (HDFs) are seeded at serially diluted densities (5-1.25×10^4 cells per well) in a 24-well plate and cultured for 3 hours. The ScienCell™ Colorimetric GAPDH Assay is then applied to the corresponding cell lysate collected. The ΔA_{340nm} of each lysate measured during a time interval of 3-12 minute 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.