

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 α -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 ($A_{340\text{nm}}/\text{min}$).

Kit Components

Cat. No.	# of vials	Name	Quantity	Storage
8148a	1	Cell Lysis Buffer	10 ml	4°C
8148b	1	GAPDH Assay Buffer	12.5 ml	4°C
8148c	1	100 mM 3-PGA	1 ml	-20°C
8148d	1	100 mM L-Cysteine	0.5 ml	-20°C
8148e	1	7 mM β -NADH	0.25 ml	-20°C
8148f	1	34 mM ATP	0.5 ml	-20°C
8148g	1	400 Units/ml 3-PGK	0.125 ml	-20°C
8148h	1	GAPDH standard (1 unit/ml)	0.25 ml	-20°C

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 $A_{340\text{nm}}$ & GAPDH concentration at different reaction time (Figure 1), $A_{340\text{nm}}$ & reaction time with different GAPDH concentrations (Figure 2), and $A_{340\text{nm}}/\text{min}$ & 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 ($5-1.25 \times 10^4$ cells per well). The resulting $A_{340\text{nm}}$ at 3-12 minutes of reaction, and the $A_{340\text{nm}}/\text{min}$ 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. 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 ($\sim 0.1\text{-}1 \times 10^5$ 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 2-8°C for 20 min with gentle agitation to lyse cells. Centrifuge the lysate at $14,000 \times g$ in pre-cooled centrifuge for 3 minutes, transfer the supernatant to fresh tube and discard the pellet. Cell lysate can be stored at -70 °C 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 $A_{340\text{nm}}$ 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 $A_{340\text{nm}}$ over an 12 minute interval, collecting data every 3 min.*

D. Calculations:

1. Subtract the measured $A_{340\text{nm}}$ at different reaction time from the initial $A_{340\text{nm}}$ to obtain the corresponding $A_{340\text{nm}}$ for each sample and GAPDH standard at different reaction time. Average the $A_{340\text{nm}}$ of replicate wells.
2. Based on the $A_{340\text{nm}}$ of the GAPDH standard solutions, two kinds of standard curves can be made: First, the standard curve of $A_{340\text{nm}}$ vs. GAPDH concentration at reaction time (e.g. Figure 1). Second, the standard curve of $A_{340\text{nm}}/\text{min}$ vs. GAPDH concentration (e.g. Figure 3), in which the $A_{340\text{nm}}/\text{min}$ is calculated by plotting the $A_{340\text{nm}}$ 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 $A_{340\text{nm}}/\text{min}$ 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 -NADH	2.5
34 mM ATP	5
400 Units/ml 3-PGK	1.25
Total	145

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

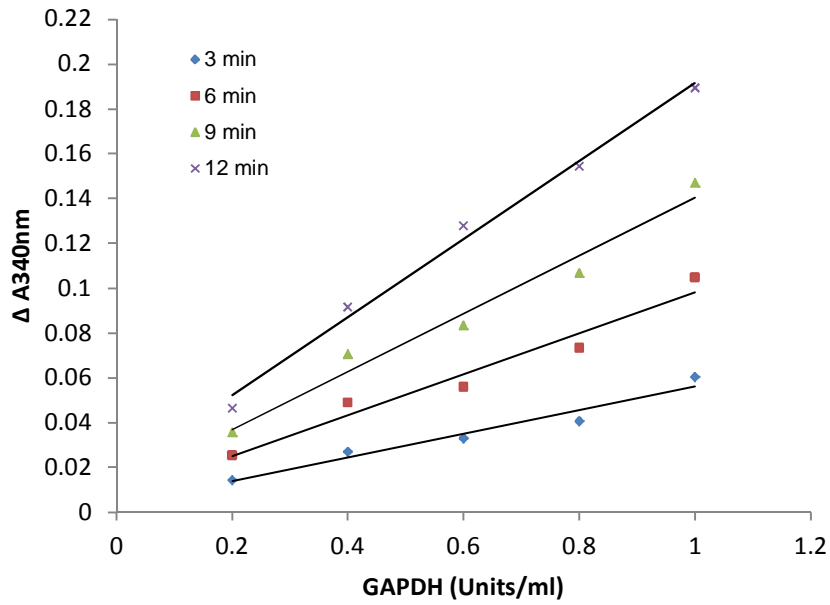


Figure 1. Standard curves of ΔA_{340nm} vs. GAPDH concentration measured after 3, 6, 9, and 12 minutes of reaction.

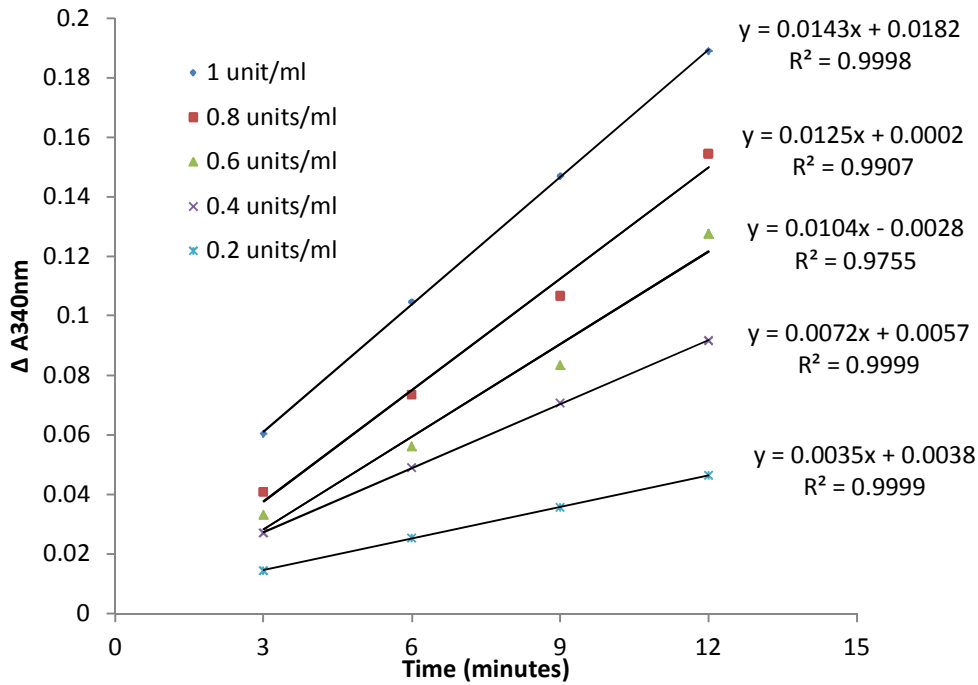


Figure 2. Standard curves of ΔA_{340nm} vs. reaction time for GAPDH solution with different concentrations.

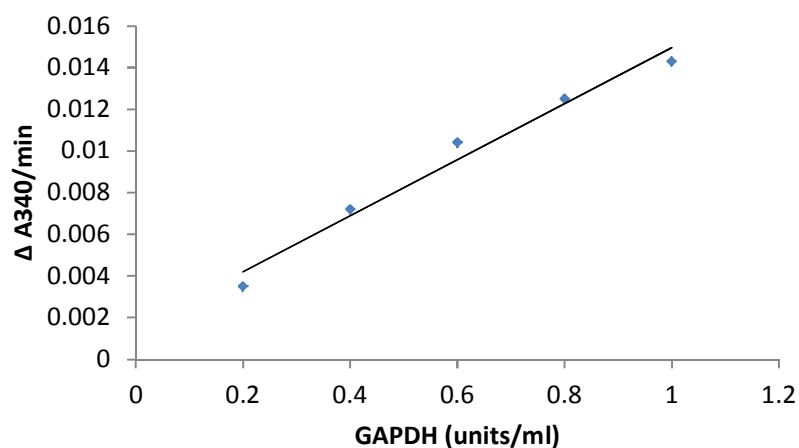


Figure 3. A standard curve of $A_{340\text{nm}}/\text{min}$ vs. GAPDH concentration, wherein the $A_{340\text{nm}}/\text{min}$ is calculated as the slope of the standard curves shown in Figure 2.

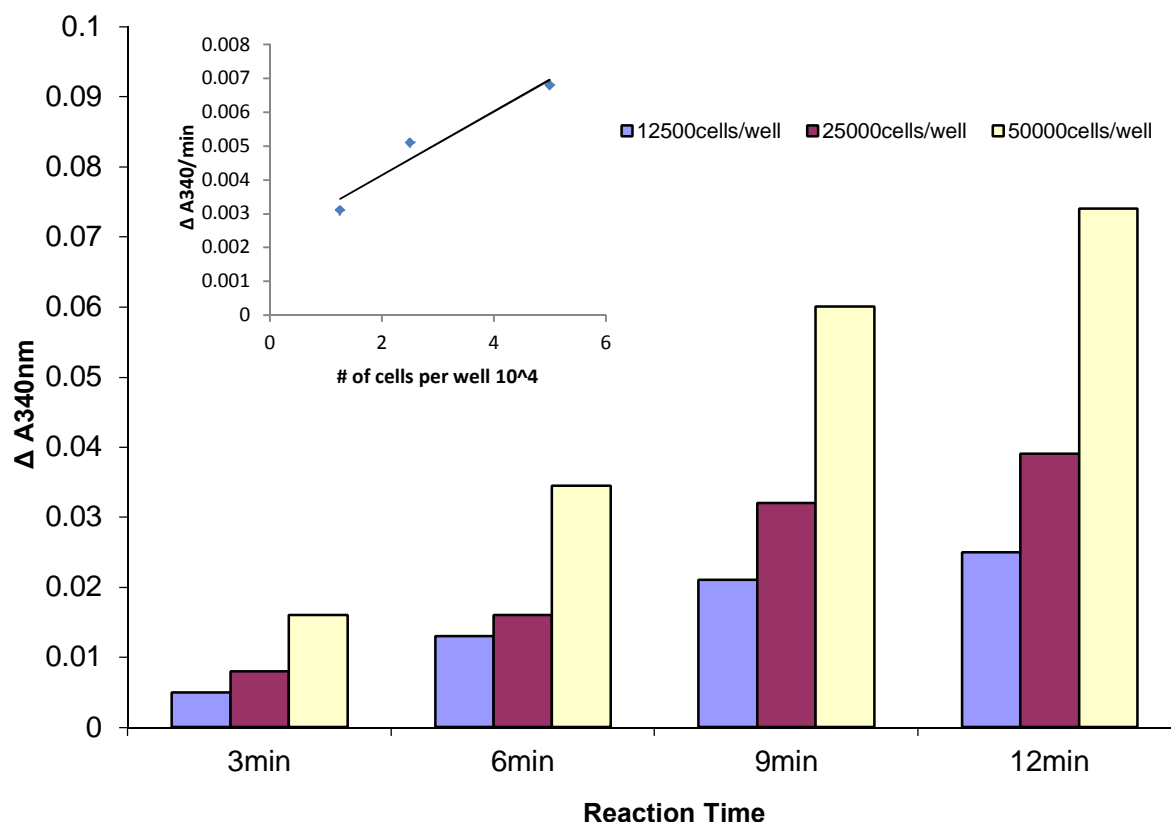


Figure 4. Human Dermal Fibroblasts (HDFs) are seeded at serially diluted densities ($5-1.25 \times 10^4$ cells per well) in a 24-well plate and cultured for 3 hours. The ScienCell[®] Colorimetric GAPDH Assay is then applied to the corresponding cell lysate collected. The $A_{340\text{nm}}$ of each lysate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