#12581 Store at -20°C

Glucose-6-Phosphate Dehydrogenase (G6PD) Activity Assay Kit

☑ 1 Kit
100 assays (96 well format)

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New 04/14

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Vial Color	Cap Color
G6PDH Assay Buffer (1X)		25 ml		
G6PDH Substrate (40X)*		250 µl	Clear	Blue
G6PDH Cofactor (100X)*		100 µl	Clear	Yellow
$NADP^+$ (100X)*		100 µl	Clear	White
G6PDH Developer (100X)*		100 µl	Amber	Amber
G6PDH Positive Control (100X)*		50 µl	Amber	Amber
PathScan® Sandwich ELISA Lysis Buffer (1X)	7018	30 ml		

**Supplied Lyophilized - Volumes shown are for reconstitution*

Description: The Glucose-6-Phosphate Dehydrogenase (G6PD) Activity Assay Kit contains the necessary reagents for rapid, sensitive, and simple detection of G6PD activity in various samples. In the assay, glucose-6-phosphate (G6P), in the presence of NADP, is oxidized by G6PD to generate 6-phosphogluconolactone and NADPH. The generated NADPH is then amplified by the diaphorase-cycling system to produce highly fluorescent resorufin molecules (see Figure 1). The relative fluorescent units (RFU) can then be determined using a plate reader with excitation about 540 nm and emission about 590 nm. The magnitude of RFU is proportional to G6PD activity in the sample.

Background: Glucose-6-phosphate dehydrogenase (G6PD) catalyses the first, and rate-limiting, step of the pentose phosphate pathway (1). The NADPH generated from this reaction is essential to protect cells from oxidative stress (1). Research studies have shown that p53 interacts with G6PD and inhibits its activity, therefore suppressing glucose consumption through the pentose phosphate pathway (2). In cancer cells with p53 mutations, the increased glucose consumption is directed towards increased biosynthesis, which is critical for cancer cell proliferation (2).

Specificity/Sensitivity: The Glucose-6-Phosphate Dehydrogenase (G6PD) Activity Assay Kit detects sample G6PD activity. The presence of NADH and NADPH may interfere with the assay.

Background References:

(1) Au, S.W. et al. (2000) *Structure* 8, 293-303.

(2) Jiang, P. et al. (2011) *Nat Cell Biol* 13, 310-6.

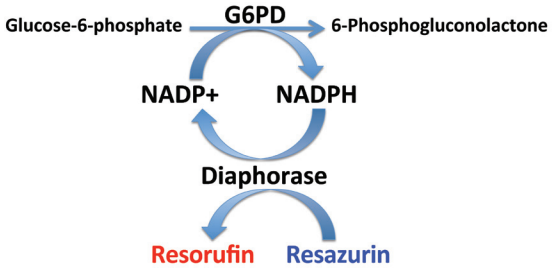

Figure 1. Schematic diagram of Glucose-6-Phosphate Dehydrogenase assay. Glucose-6-phosphate (G6P) is oxidized by G6PD in the presence of NADP, which generates 6-phosphogluconolactone and NADPH. The generated NADPH is then amplified by the diaphorase-cycling system to produce highly fluorescent resorufin molecules.

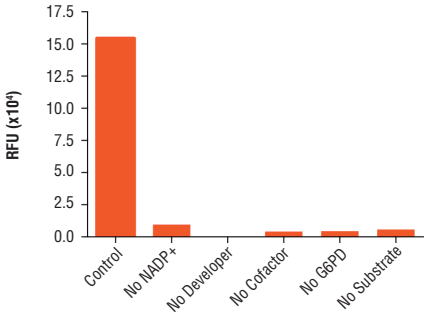

Figure 2. Each assay component is individually omitted from the assay system and the resultant RFU is compared to that of a control test that contains all of the assay components.

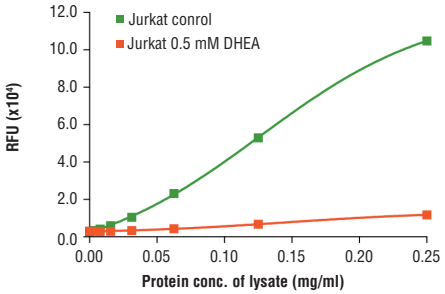

Figure 3. The relationship between the protein concentration of lysates from untreated and G6PD inhibitor DHEA (0.5 mM) treated Jurkat cells and relative fluorescence (RFU) is shown. The G6PD inhibitor DHEA can effectively inhibit this chain reaction as shown in this figure.

#12581

Assay Protocol

A Instrumentation

1. A plate reader that can read 96-well plates with excitation around 540 nm, and emission at 590 nm.
2. 96-well black plate

B Reagent Preparation

1. Please reconstitute the following lyophilized components:

Component	dH_2O Volume
G6PDH Substrate	250 µl
G6PDH Developer	100 µl
G6PDH positive control	50 µl
NADP	100 µl
G6PDH cofactor	100 µl

2. Calculate the number of tests: number of samples + positive controls.
3. Dilute **Total Detection Solution** for the calculated number of tests.
   70 µl/well is recommended for a 96-well plate
   20 µl/well is recommended for a 384-well plate.

   Number of tests = 10 samples (n=3) + 3 positive controls
   96-Well Plate: 70 µl/well x 33 samples = 2310 µl + 10% = ~2500 µl
   384-Well Plate: 20 µl/well x 33 samples = 660 µl) + 10% = ~750 µl
4. Make **Negative Control Solution** (See Table)
   Note: **DO NOT** add G6PD substrate to the **Negative Control Solution.**
5. Make **Positive Control Solution** (See Table)
6. Thaw out 1X Cell lysis buffer on ice.

Table 1: Example of calculation for 10 samples in a 96-well plate (All triplicates)

	Total Detection Solution (µl)	Negative Control Solution (µl)	Positive Control Solution (µl)
G6PD Substrate (40X)	62.5	0	0
G6PD Developer (100X)	25	3.3	0
G6PDH cofactor (100X)	25	3.3	0
G6PD positive control (100X)	0	0	1
NADP+ (100X)	25	3.3	0
G6PD Assay Buffer (1X)	2362.5	320	99
Total (µl) (With ~10% extra volume)	2500 (30 Samples + 3 Positive Controls)	330	100

Table 2: Example calculation for 10 samples in a 384-plate (All triplicates)

	Total Detection Solution (µl)	Negative Control Solution (µl)	Positive Control Solution (µl)
G6PD Substrate (40X)	19	0	0
G6PD Developer (100X)	7.5	1	0
G6PDH cofactor (100X)	7.5	1	0
G6PD positive control (100X)	0	0	1
NADP+ (100X)	7.5	1	
G6PD Assay Buffer (1X)	708.5	97	99
Total (µl) (With ~10% extra volume)	750 (30 Samples + 3 Positive Controls)	100	100

C Preparing Cell Lysates

For adherent cells

1. Grow target cells to 80–90% confluence and aspirate media.
2. Add fresh media containing regulator for desired time.
3. Aspirate media and rinse cells once with ice-cold 1X PBS (#9872).
4. Aspirate PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter).
5. Incubate the plate on ice for 5 min.
6. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
7. Sonicate lysates on ice.
8. Microcentrifuge for 10 min (x14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate.
9. Store at –80°C in single-use aliquots.

For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10^6 viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (x14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

D Assay Protocol

1. Dilute sample in G6PD Assay Buffer (1X) to desired concentration.
2. Add 70 µl of **Total Detection Solution** and 30 µl of sample in a black 96-well plate. Mix well. (Alternatively, add 20 µl of **Total Detection Solution** and 10 µl of sample for a 384-well plate.)
3. Negative control: Add 100 µl of **Negative Control Solution** to three wells. (Add 30 µl of **Negative Control Solution** for a 384-well plate.)
4. Positive control: Add 70 µl of **Total Detection Solution** and 30 µl **Positive Control** to three wells. Mix well.
   (Add 20 µl of **Total Detection Solution** and 10 µl of **Positive Control** for a 384-well plate.)
5. Incubate at 37°C for 15-30 min.
6. Read RFU on a plate reader with excitation around 540 nm and emission around 590 nm.