CyQUANT™ Cytotoxicity Assay Kit (G6PD Release Assay)

Catalog Numbers V23111

Pub. No. MAN0019029 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Cell death is often assayed using probes that enter the cell through areas of plasma membrane damage. The Invitrogen™ CyQUANT™ Cytotoxicity Assay Kit (G6PD Release Assay) provides an alternative method that monitors release of a cytosolic enzyme, glucose 6-phosphate dehydrogenase (G6PD), from damaged cells into the surrounding medium. G6PD is a ubiquitous enzyme that is part of the pentose phosphate pathway. Because G6PD generates NADPH (Persico, 1986; Zhang, 2000) it plays a crucial role in cellular antioxidant defense.

The CyQUANT™ Cytotoxicity Assay Kit (G6PD Release Assay) contains all of the enzymes and substrates needed to detect the release of G6PD from damaged and dying cells. The assay can be completed in less than an hour and is effective with samples containing as few as 500 cells.

Contents and storage

Reagents that are provided in the kit are sufficient for approximately 1,000 assays using 100-µL reaction volumes.

Contents	Amount	Storage [1]
Resazurin, MW = 251.17 (Component A)	5 × 75 µg	• –20°C • Dessicate • Protect from light
Dimethylsulfoxide (DMSO), anhydrous (Component B)	500 µL	
Reaction Mixture (Component C)	5 vials[2]	
5X Reaction Buffer (Component D) (0.50 M Tris, pH 7.5)	28 mL	
100X Cell-Lysis Buffer (Component E)	1 mL	
Absorption/emission of reaction product: 563/587 nm		

[1] Refer to label for expiration date.

[2] Each vial contains a lyophilized mixture of diaphorase, glucose 6-phosphate, and NADP+.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Fluorescence microplate reader capable of 530–560-nm excitation and 580–600-nm emission	MLS
Flat-bottom, clear 96-well plate compatible with spectrophotometry	MLS

Procedure overview

The CyQUANT™ Cytotoxicity Assay Kit (G6PD Release Assay) detects G6PD through a two-step enzymatic process that leads to the reduction of resazurin into red-fluorescent resorufin (Figure 1). The resulting fluorescence signal is proportional to the amount of G6PD released into the cell medium, and this release correlates with the number of dead cells in the sample (Figure 2). The fluorescence emission of resorufin (Abs/Em 563/587 nm) is beyond the autofluorescence of most biological samples. In addition, this assay results in lower background signals than are typically observed in lactate dehydrogenase-based assays (Figure 3).

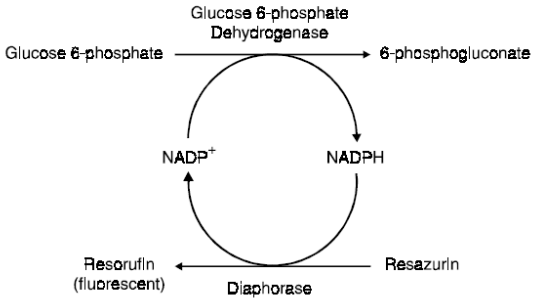

Figure 1 Principle of the coupled enzymatic assay for detection of glucose 6-phosphate dehydrogenase activity

Oxidation of glucose 6-phosphate by glucose 6-phosphate dehydrogenase results in the generation of NADPH, which leads to the reduction of resazurin by diaphorase to yield fluorescent resorufin.

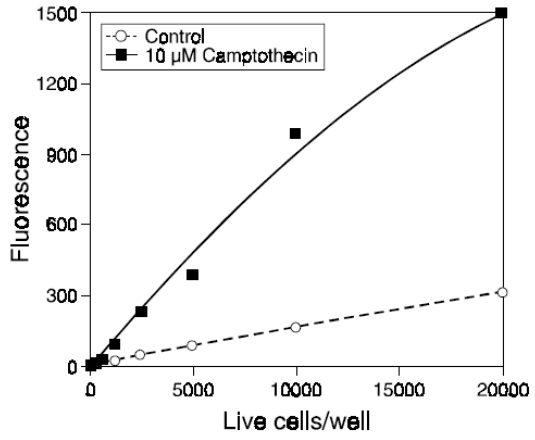

Figure 2 Detection of dead and dying cells using the CyQUANT™ Cytotoxicity Assay Kit (G6PD Release Assay)

Jurkat cells were treated with 10-µM camptothecin for six hours, then assayed for glucose 6-phosphate dehydrogenase release. An untreated control sample is shown for comparison. The fluorescence was measured in a microplate reader (excitation/emission ~530/590 nm). A background of 55 fluorescence units was subtracted from each value.

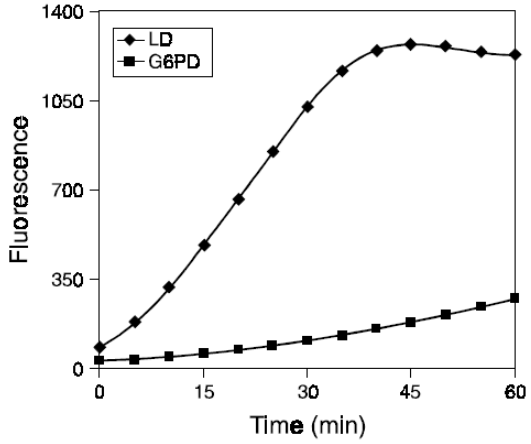

Figure 3 Comparison of signal levels due to lactate dehydrogenase and glucose 6-phosphate dehydrogenase present in serum

Bovine serum (10%) was assayed for the presence of lactate dehydrogenase (LD, diamonds) and glucose 6-phosphate dehydrogenase (G6PD, squares). G6PD was assayed using the reaction scheme shown in Figure 1; LD was detected using a similar scheme, except that in this case, LD reduces lactate to generate NADH. The result shows that the serum generates a much lower signal in the G6PD assay compared to the LD assay.

Procedural guidelines

- The protocol described in this document is designed for use with a fluorescence multiwell plate reader. If a standard fluorometer is used, adjust the volumes accordingly.
- Perform the reactions using the reaction buffer that is provided in the kit. The absorption and fluorescence of resorufin are pH-dependent. Below the pKa (~6.0), the absorption maximum of resorufin shifts to ~480 nm and the fluorescence quantum yield is markedly lower.
- Assay all samples (control and experimental) in triplicate.
- Cells can be grown in a separate plate or under different conditions, then spun down, and only the supernatant assayed. The presence of the cells themselves does not affect the assay, but under certain circumstances, it may be more convenient to assay the supernatant only.

Before you begin

Allow all kit components to warm to room temperature before use.

1. Prepare a 4-mM stock solution of Resazurin by dissolving the contents of the vial (Component A) in 75 µL of DMSO (Component B).

   Note: Store the Resazurin Stock Solution at −20°C protected from light.

2. Prepare 1X Reaction Buffer by combining 1 part 5X Reaction Buffer (Component D) with 4 parts deionized water (dH2O). Example: For 200 assays, combine 2.0 mL of 5X Reaction Buffer with 8.0 mL of dH2O.

Perform the cytotoxicity assay

1. Plate cells in a 96-well microplate (500–25,000 cells/well in a 50-µL volume). Reserve three sets of triplicate wells for the following controls: a no-cell control (medium alone), untreated cells, and completely-lysed cells.

   The completely-lysed control cells are used to determine the total amount of cellular G6PD. This control is not necessary if a quantitative determination of cell death is not needed.

   Note: High concentrations of serum will increase the background, but will not significantly impair the sensitivity of the assay.

2. Add the preferred cytotoxic agent(s) to the experimental wells, then incubate for the desired time.

   IMPORTANT! Incubations >24 hours can result in significant degradation of G6PD and impair the assay results.

3. Prepare the Reaction Mixture Solution—Reconstitute the contents of one vial of Reaction Mixture (Component C) in 400 µL of 1X Reaction Buffer. Mix the components gently. Do not vortex.

   Freeze any unused Reaction Mixture Solution in single-use aliquots.

4. Combine the following components to make a 2X Resazurin/Reaction Mixture.

   Note: Prepare only enough to add 50 µL of 2X Resazurin/Reaction Mixture to each well.

Component	Volume[1]
4-mM Resazurin Stock Solution	75 µL
Reaction Mixture Solution	400 µL
1X Reaction Buffer	9.52 mL
Note: The concentration of Resazurin in the final reaction mixture is 15 µM.	

[1] The sensitivity of the assay can be increased by reducing the Resazurin concentration in the 2X Resazurin/Reaction Mixture. For example, add 25 µL of 4-mM Resazurin and 9.57 mL of 1X Reaction Buffer to obtain a final assay concentration of 5-µM Resazurin. Note that this change decreases the maximum attainable signal level.

   Mix the components gently. Do not vortex.

5. Add 50 µL of 2X Resazurin/Reaction Mixture to each well.

6. To the completely-lysed control wells only, add 1 µL of 100X Cell-Lysis Buffer (Component E).

7. Incubate the microplate at 37°C for 10–30 minutes.

8. Measure the fluorescence (excitation 530–560 nm, emission 580–600 nm) at 5-minute intervals to determine the optimal time point for the particular experiment.

Analyze the results

1. Correct the values for the experimental cells and the completely-lysed control cells by subtracting from each the fluorescent signal of the untreated control cells.

2. For each experimental condition, determine the relative cytotoxicity by dividing the corrected fluorescence of the experimental cells by the corrected fluorescence of the completely-lysed control cells.

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References

Persico MG, Viglietto G, Martini G, Toniolo D, Paonessa G, Moscatelli C, Dono R, Vulliamy T, Luzzatto L, D'Urso M (1986) Isolation of human glucose-6-phosphate dehydrogenase (G6PD) cDNA clones: primary structure of the protein and unusual 5' non-coding region. *Nucleic Acids Res* 14(6):2511-2522.

Zhang Z, Apse K, Pang J, Stanton RC (2000) High Glucose Inhibits Glucose-6-phosphate Dehydrogenase via cAMP in Aortic Endothelial Cells. *J Biol Chem* 275(51):40042-40047.

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

Revision history: Pub. No. MAN0019029

Revision	Date	Description
A.0	2 January 2020	• Converted the legacy document (MP 23111) to the current document template, with associated updates to the publication number, warranty, trademarks, and logos. • Changed the kit name from Vybrant™ Cytotoxicity Assay Kit to CyQUANT™ Cytotoxicity Assay Kit (G6PD Release Assay).