CyQUANT® NF Cell Proliferation Assay Kit

Table 1. Contents and Storage Information

Material	Amount	Concentration	Storage*	Stability
CyQUANT® NF Cell Proliferation Assay Kit (C35006)				
CyQUANT® NF dye reagent (Component A)	220 µL	500X solution in DMSO	• Store refrigerated at ≤ 6°C • Desiccate • Protect from light	If stored correctly, the kit is stable for 6 months
Dye delivery reagent (Component B)	220 µL	500X solution in DMSO		
5X HBSS buffer (Hank's balanced salt solution) (Component C)	22 mL	NA		
CyQUANT® NF Cell Proliferation Assay Kit (C35007)				
CyQUANT® NF dye reagent (Component A)	44 µL	500X solution in DMSO	• Store refrigerated at ≤ 6°C • Desiccate • Protect from light	If stored correctly, the kit is stable for 6 months
Dye delivery reagent (Component B)	44 µL	500X solution in DMSO		
5X HBSS buffer (Hank's balanced salt solution) (Component C)	4.5 mL	NA		

* If frozen, avoid freeze-thaw cycles.

Number of Assays: 1000 for C35006 and 200 for 35007.

Spectral Data: CyQUANT® NF dye bound to DNA: 480/520 nm.

Introduction

Methods for cell proliferation analysis are generally based on the incorporation of thymidine analogs such as ^{3}H thymidine or bromodeoxyuridine (BrdU) during DNA synthesis, or on measurement of metabolic activity indices such as oxidoreductase activity or ATP levels. The CyQUANT® NF assay is based on measurement of cellular DNA content via fluorescent dye binding. Because cellular DNA content is highly regulated, it is closely proportional to cell number. The extent of proliferation is determined by comparing cell counts for samples treated with drugs or other compounds of interest with untreated controls.[1,2] The assay does not require the use of radioisotopes, enzymes, or antibodies and is not dependent on physiological activities that may exhibit cell number–independent variability. The original CyQUANT® assay[3] provides sensitive detection of cells over a 1000-fold linear dynamic range. In this assay, a freeze-thaw cell lysis step is required to facilitate the interaction of the CyQUANT® GR dye with nuclear DNA. The CyQUANT® NF assay avoids this freeze-thaw step by using a DNA binding dye in combination with a plasma membrane permeabilization reagent. The

CyQUANT® NF protocol requires only aspiration of growth medium (for adherent cells), replacement with dye binding solution, incubation for 30–60 minutes, and then measurement of fluorescence in a microplate reader. The assay is designed to produce a linear analytical response from at least 100–20,000 cells per well in most cell lines in a 96-well microplate (Figure 1).

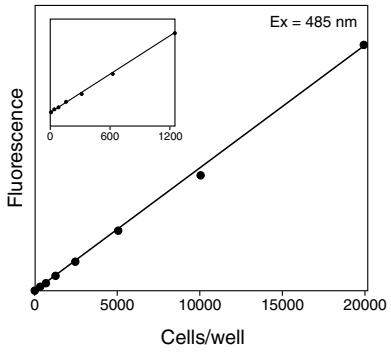

Figure 1. Quantitation of CHO cells using the CyQUANT® NF Cell Proliferation Assay Kit. CHO cells (M1WT3 ATCC CRL-1985) were plated at densities of 0–20,000 per well in a 96-well poly-D-lysine-coated microplate (Greiner Bio-One). Cells were incubated with CyQUANT® NF reagent for 30 minutes at 37°C according to the standard adherent cell analysis protocol described in this product information sheet. Fluorescence intensities of quadruplicate samples were measured with a fluorescence microplate reader using excitation at 485 ± 10 nm and fluorescence detection at 530 ± 15 nm. Plotted data points represent averages of quadruplicate samples without background subtraction. Error bars represent ± 1 standard deviation. The plotted line is a linear regression fit of all data points ($R^2 = 0.999$). The inset shows the measurement range from 0–1250 cells per well.

Before You Begin

Development of the CyQUANT® NF Assay

The CyQUANT® NF Assay for adherent cells was tested using HEK 293 (GripTite™ 293 MSR, Invitrogen), HeLa, HepG2, 3T3, and CHO (M1WT3, Figure 1) cells. The nonadherent cell protocol was developed using Jurkat T-lymphocytes. Development of both assays was performed using 96-well microplates with 100 µL of dye binding solution per well. The protocol can be adapted for 384-well microplates (see *Using 384-Well Microplates*, below).

Using 384-Well Microplates

To adapt the protocol provided in this manual for use in 384-well microplates, prepare the same total volume of dye binding solution (see step 1.2 of *Adherent Cells* and step 2.2 for *Nonadherent Cells*) and reduce the amount added per well by a factor of 4. For *Adherent Cells*, add 25 µL of 1X dye binding solution to each well instead of 100 µL (step 1.5). For *Nonadherent Cells*, add 12.5 µL of cells to each well instead of 50 µL (step 2.3) and 12.5 µL of 2X dye binding solution to each well instead of 50 µL (step 2.4).

Experimental Protocols

Adherent cells

1.1 Prepare 11 mL of 1X HBSS buffer by diluting 2.2 mL of 5X HBSS buffer (Component C) with 8.8 mL of deionized water.

1.2 Prepare 1X dye binding solution by adding 22 µL of CyQUANT® NF dye reagent (Component A) to 11 mL of 1X HBSS buffer.

1.3 Plate cells in a microplate at density of 100–10,000 cells per well. Allow at least 4 hours for adhesion before proceeding to the next step in the protocol. The specification of a 4-hour cell adhesion period is given for initial guidance in setting up the assay.

Note: If a standard curve of fluorescence intensity versus cell number is required (see for example, Figure 1), determine the culture density before plating using a hemocytometer or particle counter.

1.4 Remove growth medium from cells by gentle aspiration using a manual or robotic multichannel pipettor.

Note: It is important that removal of the growth medium does not cause detachment and loss of cells. Test compounds that impair adhesion may cause underestimation of cell numbers.

1.5 Dispense 100 µL of 1X dye binding solution (prepared in step 1.2) into each microplate well using a manual or robotic multichannel pipettor.

Note: Smaller volumes of dye binding solution can be used, according to user preference. For example, we have reduced the volume to 50 µL per well in some experiments without observing significant performance deterioration.

1.6 Cover the microplate and incubate at 37°C for 30–60 minutes. This incubation period is required for equilibration of dye–DNA binding, resulting in a stable fluorescence endpoint. For small cells such as CHO and 3T3, this period can be as short as 10 minutes. For larger cells (e.g., HeLa), >30 minutes of incubation is typically required. The specification of 30–60 minutes incubation is given for initial guidance in setting up the assay.

Note: To optimize the incubation time for a particular cell type, measure fluorescence intensity as a function of time after dye addition on a cell sample at the upper end of the assay range (e.g., 10,000 cells/well). After an initial rapid increase, the fluorescence intensity should reach a stable plateau (<1% per minute change in intensity reading). The incubation time required for equilibration of dye–DNA binding may also be reduced by increasing the concentration of dye delivery reagent. For example, add 22 µL of dye delivery reagent (Component B) to the 22 µL of CyQUANT® NF dye reagent (Component A) in the dye binding solution (Step **1.2**). Note that increasing the dye delivery reagent concentration may increase the cell-free background signal, resulting in some loss of sensitivity at low cell numbers.

1.7 Measure the fluorescence intensity of each sample using a fluorescence microplate reader with excitation at ~485 nm and emission detection at ~530 nm. Note that the stable fluorescence intensity endpoint typically persists for at least 2 hours after equilibration (Step 1.6), providing some flexibility in scheduling the fluorescence measurements in experiments involving multiple assay plates. Fluorescence measurements may be performed at 37°C or at ambient temperature (typically ~22°C).

Nonadherent cells

2.1 Prepare 11 mL of 1X HBSS buffer by diluting 2.2 mL of 5X HBSS buffer (Component C) with 8.8 mL of deionized water.

2.2 Prepare 2X dye binding solution by adding 22 µL of CyQUANT® NF dye reagent (Component A) to 5.5 mL of 1X HBSS buffer.

2.3 Sediment cells by centrifugation (e.g., 300 × g for 5–7 minutes), resuspend in 1X HBSS buffer (prepared in step 2.1), and dispense 50 µL aliquots of suspension containing 100–10,000 cells into microplate wells.

Note: If a standard curve of fluorescence intensity versus cell number is required (see for example, Figure 1), determine the culture density before plating using a hemocytometer or particle counter.

2.4 Dispense 50 µL of 2X dye binding solution (prepared in step 2.2) into each microplate well using a manual or robotic multichannel pipettor.

2.5 Cover the microplate and incubate at 37°C for 30–60 minutes. This incubation period is required for equilibration of dye–DNA binding, resulting in a stable fluorescence endpoint. The specification of 30–60 minutes incubation is given for initial guidance in setting up the assay.

Note: To optimize the incubation time for a particular cell type, measure fluorescence intensity as a function of time after dye addition on a cell sample at the upper end of the assay range (e.g., 10,000 cells/well). After an initial rapid increase, the fluorescence intensity should reach a stable plateau (<1% per minute change in intensity reading). The incubation time required for equilibration of dye–DNA binding may also be reduced by increasing the concentration of dye delivery reagent. For example, add 22 µL of dye delivery reagent (Component B) to the 22 µL of CyQUANT® NF dye reagent (Component A) in the dye binding solution (Step 1.2). Note that increasing the dye delivery reagent concentration may increase the cell-free background signal, resulting in some loss of sensitivity at low cell numbers.

2.6 Measure the fluorescence intensity of each sample using a fluorescence microplate reader with excitation at ~485 nm and emission detection at ~530 nm. Note that the stable fluorescence intensity endpoint typically persists for at least 2 hours after equilibration (Step 2.5), providing some flexibility in scheduling the fluorescence measurements in experiments involving multiple assay plates. Fluorescence measurements may be performed at 37°C or at ambient temperature (typically~22°C).

References

1. J Immunol Meth 142, 199 (1991); **2.** J Immunol Meth 212, 99 (1998); **3.** J Immunol Meth 254, 85 (2001).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
C35006	CyQUANT® NF Cell Proliferation Assay Kit *1000 assays*	1 kit
C35007	CyQUANT® NF Cell Proliferation Assay Kit *200 assays*	1 kit

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