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Cell-Quant™ No Wash Cell Proliferation Assay Kit
Catalog Number: A014

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
Cell-Quant™ Dye Reagent (Component A)	**250 µL**	**500X in DMSO**	**-20 °C**	**The product is stable for at least one year when stored as directed.**
5X HBSS buffer (Hank's balanced salt solution) (Component B)	**25 mL**	**NA**	**4 °C**	

Number of assays: 1000.
Fluorescence excitation/emission maxima, in nm: Cell-Quant™ Dye bound to DNA: 490/520.

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 Cell-Quant™ No Wash 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. 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 Cell-Quant™ No Wash assay 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.

Experimental Protocols

Note: The Cell-Quant™ No Wash Assay for adherent cells was tested using HeLa, and CHO 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.

Adherent cells

1.1 Prepare 12.5 mL of 1X HBSS buffer by diluting 2.5 mL of 5X HBSS buffer (Component B) with 10 mL of deionized water.

1.2 Prepare 1X dye binding solution by adding 25 µL of Cell-Quant™ dye reagent (Component A) to 12.5 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, determine the culture density before plating using a hemocytometer or particle counter.

1.4 Remove growth medium from cells by gentle aspiration using pipette.

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 pipette.

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.

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).

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.

Nonadherent cells

2.1 Prepare 12.5 mL of 1X HBSS buffer by diluting 2.5 mL of 5X HBSS buffer (Component B) with 10 mL of deionized water.

2.2 Prepare 2X dye binding solution by adding 25 µL of Cell-Quant™ dye reagent (Component A) to 6.25 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, 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 pipette.

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).

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.