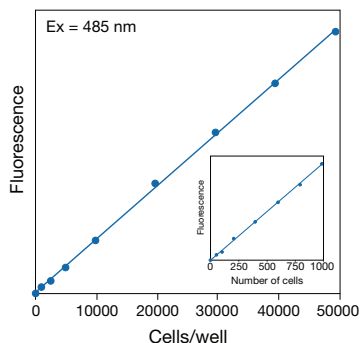


The CyQUANT® cell proliferation assay has a number of significant advantages over other proliferation assays:

- **Sensitivity and linearity.** The CyQUANT® assay is linear from 50 or fewer cells to at least 50,000 cells in 200  $\mu$ L volumes (Figure 15.4.12); increasing the dye concentration extends the linear range to at least 250,000 cells. Methods that employ Hoechst 33258<sup>66</sup> (H1398, H3569, H21491) or Hoechst 33342<sup>67</sup> (H1399, H3570, H21492) to measure cell number and proliferation are much less sensitive—detection limits of 500 cells for Hoechst 33258<sup>66</sup> or 2500 cells for Hoechst 33342<sup>67</sup>—and have much smaller effective ranges.
- **No radioactivity.** Unlike assays that measure <sup>3</sup>H-thymidine incorporation, the CyQUANT® assay does not require radioisotopes and thus does not have the hazards or the expense associated with use, storage and disposal of radioisotopes.
- **Quick and easy protocol.** The CyQUANT® assay is a single-step procedure that requires no lengthy incubation steps and can be completed within an hour (Figure 15.4.11).
- **Specificity and reliability.** The assay is specific for total nucleic acids, with essentially no interference from other cell components. No wash steps are required because cellular growth media do not significantly interfere with CyQUANT® GR fluorescence. The CyQUANT® assay is reliable for cell quantitation, even without treatment to eliminate cellular RNA. However, addition of RNase or DNase permits the easy quantitation of DNA or RNA, respectively, in the sample.
- **Convenience.** Unlike assays that use tetrazolium salts, <sup>3</sup>H-thymidine, BrdU, neutral red or methylene blue,<sup>67–70</sup> the CyQUANT® procedure is not dependent on cellular metabolism. Thus, cells can be frozen and stored prior to assaying, with no reduction in signal, or they can be assayed immediately after collection. Time-course assays are simplified because data obtained from stored samples taken at widely different time intervals can be assayed together with a single standard curve determination.

We have found the CyQUANT® Cell Proliferation Assay Kit to be useful for assaying widely disparate cell types, including:

- Human neonatal fibroblasts, keratinocytes, melanocytes, umbilical vein endothelial cells (HUVEC) and dermal microvascular endothelial cells (DMVEC)



**Figure 15.4.12** Quantitation of NIH 3T3 fibroblasts using the CyQUANT® Cell Proliferation Assay Kit (C7026). Fluorescence measurements were made using a microplate reader with excitation at 485 nm and emission detection at 530 nm. The linear range of the assay under these conditions is from 50 to 50,000 cells per 200  $\mu$ L sample. The inset shows the linearity that can be obtained at very low numbers of cells.

- Murine fibroblasts (NIH 3T3 and CRE BAG 2 cells) and myeloma (P3X63A68) cells
- Madin–Darby canine kidney (MDCK) cells
- Chinook salmon embryo (CHSE) cells
- Rat basophilic leukemia (RBL) and glioma (C6) cells

Determination of total cell number using the CyQUANT® GR reagent is potentially useful for quantitating cell adhesion (see “Cell Adhesion” in Section 15.6) and for determining the total number of cells in a tissue. Each CyQUANT® Cell Proliferation Assay Kit (C7026) includes:

- CyQUANT® GR reagent
- Cell-lysis buffer
- DNA standard for calibration
- Detailed protocols

The kit supplies sufficient materials for performing 1000 assays based on a 200  $\mu$ L sample volume or a proportionately lower number of assays with a larger sample volume. The CyQUANT® cell-lysis buffer (a 20X concentrate, C7027) is also available separately and has been formulated to produce efficient lysis, to protect nucleic acids from nuclease activity and to dissociate proteins that may interfere with dye binding to nucleic acids. It may prove generally useful in the development of other assays that require cell lysis.

### CyQUANT® NF Cell Proliferation Assay Kit

The CyQUANT® NF Cell Proliferation Assay Kit provides a fast and sensitive method for counting cells in a population and measuring proliferation in microplate format.<sup>71</sup> This assay can be completed in 1 hour, with no washes, cell lysis, long incubations or radioactivity required, and it is not dependent on physiological activities that may exhibit cell number-independent variability. The CyQUANT® NF assay eliminates the freeze-thaw cell lysis step of the original CyQUANT® cell proliferation assay by using a cell-permeant DNA-binding dye in combination with a plasma membrane-permeabilization reagent. The CyQUANT® NF 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 CyQUANT® NF assay has a linear detection range from at least 100 to 20,000 cells per well in most cell lines using a 96-well microplate format and a 100  $\mu$ L assay volume.

The CyQUANT® NF Cell Proliferation Assay Kit can be used with either a 96-well or 384-well microplate format and is available in two configurations: a 200-assay kit (C35007) and a 1000-assay kit (C35006) for high-throughput applications.

Each kit contains:

- CyQUANT® NF dye reagent
- Dye delivery reagent
- Concentrated Hank’s balanced salt solution (HBSS)
- Detailed protocols

### CyQUANT® Direct Cell Proliferation Assay Kit

CyQUANT® Direct Cell Proliferation Assay is a fluorescence-based proliferation and cytotoxicity assay for microplate readers. The no-wash, homogeneous format and fast add-mix-read protocol makes the CyQUANT® Direct assay ideal for high-throughput screening (HTS) applications. The assay can be completed in 1 hour, with no washes, cell