Quantos™ Cell Proliferation Assay Kit

INSTRUCTION MANUAL

Catalog #302011

Revision #041001a

For In Vitro Use Only

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Stratagene. Stratagene shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

United States and Canada

Stratagene
11011 North Torrey Pines Road
La Jolla, CA 92037
Telephone (858) 535-5400
Order Toll Free (800) 424-5444
Technical Services (800) 894-1304
Internet tech_services@stratagene.com
World Wide Web www.stratagene.com

Stratagene European Contacts

Location	Telephone	Fax	Technical Services
Austria	0800 312 526	0800 312 527	017 956 7036
Belgium	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 96078	0800 96024	027 13 12 11
France	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 100391	0800 881323	
Germany	00800 7000 7000	00800 7001 7001	00800 7400 7400
	06221 400 634	06221 400 639	06221 400 637
	0130 840 911	0130 762 088	069 9509 6197
Netherlands	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 023 0446	0800 023 0447	0800 023 0448
Switzerland	00800 7000 7000	00800 7001 7001	00800 7400 7400
	061 6930 540	061 6930 544	01 800 9045
	0800 830 250	0800 825 225	
United Kingdom	00800 7000 7000	00800 7001 7001	00800 7400 7400
	01223 420 955	01223 420 234	0171 365 1056
	0800 585 370	0800 783 0889	

All Other Countries

Please contact your local distributor. A complete list of distributors is available at *www.stratagene.com*.

Quantos™ Cell Proliferation Assay Kit

CONTENTS

Quantos™ Cell Proliferation Assay Kit

MATERIALS PROVIDED

Material provided[a]	Quantity	Storage condition
Dye reagent concentrate (1000×)	0.1 ml	–20°C
Cell lysis buffer	100 ml	4°C

[a] Sufficient reagents are provided to perform 1000 microassays in 96-well microtiter plates.

Caution *Wear gloves when handling the dye reagent because the dye is a suspected mutagen.*

STORAGE CONDITIONS

Dye Reagent Concentrate: –20°C

Cell Lysis Buffer: 4°C

ADDITIONAL MATERIALS REQUIRED

Fluorometer that reads either microtiter plates or cuvettes
96-well microtiter plates or cuvettes
Hemacytometer

Revision #041001a

INTRODUCTION

Stratagene's Quantos™ cell proliferation assay kit is a system for accurately quantitating the number of cells in a culture. The cell proliferation assay produces accurate counts of either adherent or suspension cells for a wide range of cell concentrations and experimental conditions. The Quantos assay kit can also be used to measure the amount of double-stranded DNA in a sample of plasmid, lambda, or genomic DNA.

The cell proliferation assay is performed by lysing the cells in a sample and incubating the cell lysate with a dye that fluoresces when bound to DNA. The intensity of the fluorescence from the DNA–dye complex is then measured with a standard fluorometer. The number of cells in the sample is determined by comparing the fluorescence intensity of the sample to a previously generated standard curve. The standard curve relates the number of cells in a sample of the specific cell type of interest to the intensity of the fluorescence from the DNA–dye complex.

The Quantos cell proliferation assay kit is useful as a high-throughput-screening method because a large number of samples can be analyzed quickly in the 96-well format. The Quantos kit is ideal for quantitating cell proliferation during the course of an experiment because samples collected at many time points during an experiment can be analyzed quickly.

PREPROTOCOL CONSIDERATION

The quantities of the reagents given in the following protocols are optimized for a 96-well microtiter plate. See the following table for the volumes of 1× dye reagent recommended for several different types of tissue culture plasticware and two sizes of cuvettes.

Assay vessel	Volume of 1× dye reagent (µl/well or cuvette)
96-well microtiter plate	100
48-well microtiter plate	200
24-well microtiter plate	500
12-well microtiter plate	1000
6-well microtiter plate	2500
50-µl cuvette	50
1-ml cuvette	1000

PREPARING THE REAGENTS

Caution *Wear gloves when handling the dye reagent because the dye is a suspected mutagen.*

Note *For optimal results, prepare a sufficient quantity of the 1× dye reagent on the day of the assay. Each microassay in a 96-well microtiter plate requires 100 µl/well of 1× dye reagent.*

Prepare the 1× dye reagent by diluting the 1000× dye reagent concentrate 1000-fold with the cell lysis buffer (i.e., add 1 part of dye reagent concentrate to 999 parts of the cell lysis buffer).

GENERATING A STANDARD CURVE

The standard curve is a graph of the mathematical relationship between the number of cells in a sample and the intensity of the fluorescence from the DNA–dye complex (see Figure 1). The relationship between the fluorescence intensity and the number of cells is linear for up to 100,000 cells/well or greater, depending on the cell type (in 100-µl suspensions). The absolute fluorescence intensity of the DNA–dye complex differs for different cell types. Therefore, a standard curve must be generated for the specific cell type of interest.

A standard curve is generated by measuring the fluorescence intensity of the DNA–dye complex of several standards that contain different numbers of lysed cells. The fluorescence intensity vs. the number of cells for each standard is plotted, and a regression line through the data points is determined using linear regression analysis.

Protocols for Generating a Standard Curve

Two protocols for generating a standard curve are provided. Using *Protocol 1: Lysing Cell Pellets*, a suspension of cells is pelleted and lysed, then the cells are plated and assayed. Using *Protocol 2: Lysing Plated Cells*, the cells are plated first, then the cells are lysed and assayed. Either protocol can be used to generate a standard curve for both adherent and suspension cells. To choose which protocol to use, consider the following:

- *Protocol 1 is the easiest and quickest to perform.*
- *Both protocols are equally accurate for suspension cell types.*
- *For adherent cell types, protocol 2 is significantly more accurate than protocol 1.*

Note *Stratagene recommends running the standards in triplicate in a 96-well microtiter plate.*

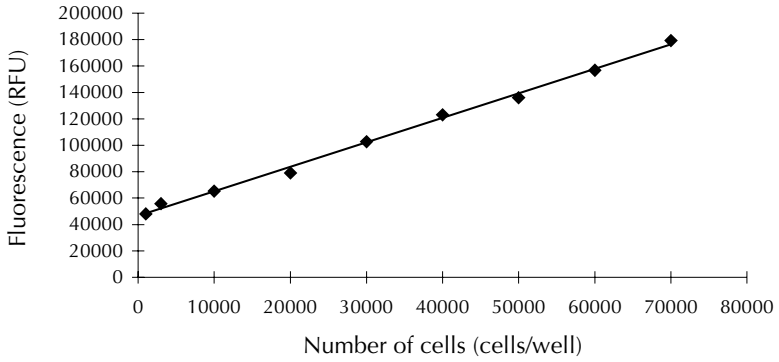

FIGURE 1 A standard curve generated with LNCaP cells; *RFU*, relative fluorescence units. The equation of the regression line is $y=mx+b$, where y is the fluorescence intensity, m is the slope of the line, b is the y-intercept, and x is the number of cells in the sample. The fluorescence was detected by a multi-plate fluorometer. The excitation wavelength was 355 nm, and the emission wavelength was 460 nm.

Protocol 1: Lysing Cell Pellets

1. Prepare a suspension of cells.

2. Count the cells using a hemacytometer.

3. Transfer 1×10^6 cells to a centrifuge tube and spin the cells for 5 minutes at 200 × g to pellet the cells.

4. Carefully remove the supernatant.

5. Freeze the cell pellet at –70°C (or below) for 15 minutes.

6. Thaw the cell pellet at room temperature.

7. Add 1 ml of the 1× dye reagent to the cells and resuspend the cells using a vortex mixer. The cell concentration should now be 1000 cells/µl.

8. Add the cell suspension to the wells of a 96-well microtiter plate in aliquots of 1, 5, 10, 20, 30, 40, 50, 60, and 70 µl/well (a range of 1000–70,000 cells/well).

9. Using the reverse-pipetting technique to minimize formation of air bubbles (see *Appendix: Reverse-Pipetting Technique*), increase the final volume in each well to 100 •µl with 1× dye reagent (i.e., add 99, 95, 90, 80, 70, 60, 50, 40, and 30 µ•l/well, respectively).

10. Incubate the microtiter plate for 1 hour at room temperature.

11. Read the plate using a microtiter plate-reading fluorometer with filters appropriate for 355-nm excitation and 460-nm emission.

12. Plot the fluorescence intensity vs. the number of cells for each standard and determine the regression line through the data points.

Protocol 2: Lysing Plated Cells

1. Prepare a suspension of cells.

2. Count the cells using a hemacytometer.

3. Aliquot various volumes of the cell suspension into the wells of a 96-well microtiter plate within the range of 1000–100,000 cells/well.

   Note *For adherent cell types, the maximum cell density (number of cells/cm^2) at confluence must be determined experimentally. The maximum number of cells per well at confluence in a 96-well plate can then be calculated (maximum number of cells/well = cells/cm^2 × 0.3 cm^2/well). Many adherent cell types cannot achieve cell densities greater than 50,000 cells/well in a 96-well plate, so adjust the range of the standard curve appropriately to reflect the cell type used.*

4. For adherent cells, incubate the plate for 4–18 hours (the duration depends on the cell type) to allow the cells to attach to the surface of the plate. Monitor the cells under a microscope during the incubation to observe when they are attached to the plate. Stop the incubation after the cells attach to the plate but before the cells undergo mitosis.

   Note *The duration of the incubation is critical. If the plate incubates longer than the time required for the cells to attach, the cells may undergo mitosis, resulting in an increase in the quantity of DNA and the number of cells in the sample.*

5. For adherent cells, carefully decant or aspirate the medium from the wells.

   For suspension cells, spin the microtiter plate in a centrifuge at 200 × g for 5 minutes to pellet the cells. **Carefully** remove the medium from the microtiter plate by inverting and blotting the plate. If the cell pellets dislodge easily, **carefully** aspirate the supernatant from each well, leaving a small amount of medium (~50 µl) in each well to prevent the accidental aspiration of cells from the pellet.

   Caution *Be careful while removing the medium. It is easy to accidentally blot or aspirate the cells from the wells.*

6. Freeze the plate at –70°C (or below) for 15 minutes.

7. Thaw the plate at room temperature.

8. Add 100 µl of 1× dye reagent to each well using the reverse-pipetting technique to minimize the formation of air bubbles (see *Appendix: Reverse-Pipetting Technique*).

9. Incubate the microtiter plate for 1 hour at room temperature.

10. Read the plate using a microtiter plate-reading fluorometer with filters appropriate for 355-nm excitation and 460-nm emission.

11. Plot the fluorescence intensity vs. the number of cells for each standard and determine the regression line through the data points.

CELL PROLIFERATION ASSAYS

Two protocols for the cell proliferation assay are provided: one is for adherent cells and the other is for suspension cells.

Cell Proliferation Assay for Adherent Cells

Note *For adherent cell types, the maximum cell density (number of cells/cm^2) at confluence must be determined experimentally. The maximum number of cells per well at confluence in a 96-well plate can then be calculated (maximum number of cells/well= cells/cm^2 × 0.3 cm^2/well). Many adherent cell types cannot achieve cell densities greater than 50,000 cells/well in a 96-well plate, so adjust the range of the standard curve appropriately to reflect the cell type used.*

1. Carefully aspirate or decant the medium from the wells of the 96-well microtiter plate.

2. Freeze the plate at –70°C (or below) for 15 minutes.

3. Thaw the plate at room temperature.

4. Using the reverse-pipetting technique (see *Appendix: Reverse-Pipetting Technique*) to minimize the formation of air bubbles, add 100 µl of 1× dye reagent to each well.

5. Incubate the microtiter plate for 1 hour at room temperature.

6. Read the plate using a microtiter plate-reading fluorometer with filters appropriate for 355-nm excitation and 460-nm emission.

7. Calculate the number of cells in the sample by substituting the fluorescence intensity of the sample into the equation of the regression line generated for the specific cell type assayed.

Cell Proliferation Assay for Suspension Cells

1. Centrifuge the cells for 5 minutes at 200 × g to pellet the cells.

2. **Carefully** remove the medium from the wells of the 96-well microtiter plate by inverting and blotting the plate. If the cell pellets dislodge easily, **carefully** aspirate the medium from each well, leaving a small amount of medium (~50 µl) in each well to prevent the accidental aspiration of the cells in the pellet.

   Caution *Be careful while removing the medium. It is easy to accidentally blot or aspirate the cells from the wells.*

3. Freeze the plate at –70°C (or below) for 15 minutes.

4. Thaw the plate at room temperature.

5. Using the reverse-pipetting technique (see *Appendix: Reverse-Pipetting Technique*) to minimize the formation of air bubbles, add 100 µl of 1× dye reagent to each well.

6. Incubate the microtiter plate for 1 hour at room temperature.

7. Read the plate using a microtiter plate-reading fluorometer with filters appropriate for 355-nm excitation and 460-nm emission.

8. Calculate the number of cells in the sample by substituting the fluorescence intensity of the sample into the equation of the regression line generated for the specific cell type assayed.

QUANTITATING THE AMOUNT OF DNA IN A SAMPLE

Generating a Standard Curve

1. Beginning with double-stranded DNA of a known concentration, prepare several different concentrations of DNA within the range of 0.5–10 µg/ml by serially diluting the DNA with 1× dye reagent.

2. Add 100 µl of each serial dilution of DNA to the wells of a microtiter plate.

3. Incubate the microtiter plate for 1 hour at room temperature.

4. Read the plate using a microtiter plate-reading fluorometer with filters appropriate for 355-nm excitation and 460-nm emission.

5. Plot the fluorescence intensity vs. the quantity of DNA for each standard and determine the regression line through the data points.

DNA Quantitation Assay

1. Add 1–10 µl of the sample of double-stranded DNA to each well of a microtiter plate.

2. Using the reverse-pipetting technique (see *Appendix: Reverse-Pipetting Technique*) to minimize formation of air bubbles, increase the final volume in each well to 100 µl with 1× dye reagent (i.e., add 90–99 µl/well).

3. Incubate the microtiter plate for 1 hour at room temperature.

4. Read the plate using a microtiter plate-reading fluorometer with filters appropriate for 355-nm excitation and 460-nm emission.

5. Calculate the quantity of DNA in the sample by substituting the fluorescence intensity of the sample into the equation of the regression line generated for the DNA.

TROUBLESHOOTING

Observation	Suggestion
No fluorescence	Check the fluorometer for a malfunction
	Verify that the filter set used is appropriate for 355-nm excitation and 460-nm emission
Low fluorescence intensity	Cell lysis may be incomplete; repeat the freeze–thaw step
	Increase the sensitivity setting of the fluorometer
Fluorescence intensity is off-scale	Reduce the sensitivity setting of the fluorometer
Fluorescence intensity of the DNA sample is higher than that of the standard of highest concentration	Concentration of DNA in the sample is too high. Dilute the DNA sample with 1× dye reagent
Precipitate in the cell lysis buffer	A precipitate may form during cold storage. Warm the solution to room temperature, swirl the solution gently until the precipitate disappears, then use the buffer as directed in the manual

APPENDIX: REVERSE-PIPETTING TECHNIQUE

1. Press the pipettor push button all the way down to the second stop.

2. Immerse the tip in the liquid and slowly release the push button to the full up position.

3. To dispense the liquid, press the push button to the **first stop**. Withdraw the tip from the receiving vessel without dispensing the liquid remaining in the tip.

4. To pipet multiple samples with the reverse-pipetting technique, keep the button at the first stop position after dispensing each sample. Aspirate the next sample by releasing the button to the full up position. Continue with step 3.

   Note *When finished pipetting all the samples, return the liquid remaining in the tip to the original sample container by pushing the button to the second stop position.*

ENDNOTES

Quantos is a trademark of Genetic Applications LLC.