

FluoReporter[®] Colorimetric Cell Protein Assay Kit (F-2961)

Introduction

Our FluoReporter[®] Colorimetric Cell Protein Assay Kit is based on an electrostatic dye-binding method for measuring protein content of trichloroacetic acid (TCA)-fixed cells, a method used at the National Cancer Institute (NCI) for high-throughput screening of new chemotherapeutic reagents.¹⁻³ This kit enables researchers to rapidly quantitate numbers of adherent or nonadherent cells based on protein content. All manipulations are carried out in microplate wells and can be completed in less than four hours. The FluoReporter Colorimetric Cell Protein Assay Kit (F-2961) contains the anionic xanthene dye sulforhodamine B, which forms an electrostatically stabilized complex with basic amino acid residues under moderately acidic conditions. The protein-dye complex (absorption maximum ~565 nm) can then be detected spectrophotometrically after removal of unbound dye from TCA-fixed cells. This FluoReporter assay is linear for determining protein content of 2000 to 200,000 mouse myeloma P3X cells. Because sulforhodamine B is fluorescent, the protein-dye complex can also be measured with a fluorescence microplate reader using excitation/emission maxima of ~485/590 nm, which provides suboptimal excitation but produces a linear relationship between fluorescence intensity and cell number.

Materials

Contents and Storage

- 1.04 g sulforhodamine B
- 20 mL of concentrated solubilization solution

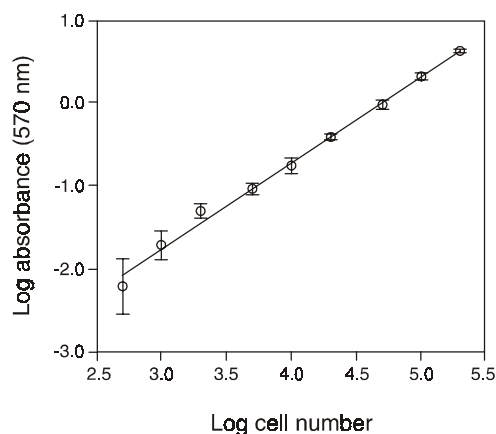


Figure 1. Analysis of mouse myeloma P3X cells using standard assay protocol. Cell numbers between 200,000 and 500 per well were measured using a Dynatech MR 600 UV microplate reader at 570 nm. Error bars indicate the standard deviations for 6 replicate measurements.

The contents are sufficient for ~2500 microplate-well assays following the standard protocols (see *Experimental Protocols*). The solubilization reagent should be stored in a refrigerator until required. The solid dye sample may be stored at room temperature.

Materials Required but Not Provided

- Microplates with a capacity of ≥ 250 μ L per well
- Microplate absorbance reader capable of readout between 550 and 580 nm
- Glacial acetic acid
- Trichloroacetic acid (see *TCA Fixation Solution*)
- Hemocytometer

Preparation

TCA Fixation Solution

The fixing solution is 80% (w/v) trichloroacetic acid (TCA). 125 mL of this reagent is sufficient to fix 2500 microplate wells of non-adherent cell suspension. Adherent cells require lower TCA concentrations (see *Staining of Non-Adherent Cells*, note **D**). TCA can be purchased from Sigma Chemical Co. as a 100% (w/v) solution (Sigma catalog number 490-10). Dilution of 100 mL of this reagent with 25 mL of water yields 125 mL of 80% TCA. Alternatively, dissolve 100 g of solid TCA in 125 mL of distilled water.

1% Acetic Acid Solution

This solution is used both to prepare the staining solution (see *SRB Staining Solution*) and as a wash reagent (Step 8). To prepare 1% acetic acid, slowly add 10 mL glacial acetic acid to 990 mL distilled water. Several (5–7) liters of this reagent will typically be required to run all the samples provided for by this kit, primarily for the washing step.

SRB Staining Solution

Dissolve the entire contents of the sulforhodamine B dye container (see *Contents and Storage*) in 260 mL of 1% acetic acid (see *1% Acetic Acid Solution*) to yield 0.4% (w/v) SRB (about 7.2 mM). This reagent should be stored in the dark at room temperature.

Solubilization Reagent

Add the entire supplied amount (20 mL) of concentrated solubilization reagent (see *Contents and Storage*) to 500 mL of distilled water. The resulting volume of solution (520 mL) is sufficient to treat all the samples provided for by this kit (2500). Store the dilute solubilization reagent in a refrigerator.

Table 1. Relative absorbance (A) of SRB at 5 nm wavelength (λ) intervals.

λ (nm)	A	λ (nm)	A	λ (nm)	A	λ (nm)	A
495	0.09	520	0.31	545	0.48	570	0.92
500	0.11	525	0.34	550	0.61	575	0.71
505	0.15	530	0.35	555	0.76	580	0.45
510	0.20	535	0.36	560	0.93	585	0.25
515	0.25	540	0.40	565	1.00	590	0.12

For example, $A_{570\text{ nm}} = A_{530\text{ nm}} \times (0.92/0.35)$

Experimental Protocols

Construction of a Standard Curve

To construct a standard curve for a particular cell type, follow the standard protocol using serial dilutions of a suspension of known cell number density determined with a hemacytometer.

Staining of Non-Adherent Cells

1. Wash cells in serum-free medium (note **A**) and resuspend to a concentration of $\sim 10^6$ cells/mL.
2. Serially dilute cells into microplate wells (note **B**) to densities in the range of 1000 to 100,000 cells in 200 μ L. 200 μ L control samples of cell free medium may be used for absorbance blank readings.
3. Gently layer 50 μ L of 80% (w/v) trichloroacetic acid (note **C**) onto each 200 μ L cell sample (final TCA concentration of 16% (note **D**)).
4. Store plates in the refrigerator for 1 hour.
5. Wash cells 3–4 times with tap water, flicking the plates vigorously to remove excess liquid between washes. Water may simply be poured over the plate from a beaker.
6. Dry plates in air (if overnight storage is desired), or in oven for about 30 minutes at 45–50°C.
7. Add enough SRB staining solution (see *SRB Staining Solution*) to completely cover the bottom of the wells (100 μ L per well is usually sufficient for a standard 96-well plate) and incubate for 30 minutes in the dark at room temperature.
8. Repeat wash step (Step 5) using 1% acetic acid in place of tap water.
9. Air- or oven-dry the samples as described above (Step 6); plates may also be stored at this point (note **E**).
10. Prepare the plate for reading by adding 200 μ L of diluted solubilization reagent (see *Solubilization Reagent*). To ensure complete dissolution of the dye, mix by repipetting or by gentle agitation of the plate for a few minutes.
11. Proceed to *Spectrophotometric Readout*.

Spectrophotometric Readout

Measure absorbance using a suitable spectrophotometric microplate reader at a wavelength between 560 and 580 nm (the absorption maximum of SRB in the solubilization reagent is 565 nm). For large numbers of cells ($>100,000$) the absorbance will typically exceed the linear instrumental range. In such cases, a second set of readings at a wavelength where the molar absorptivity of the dye is lower can be used to complete the analytical curve.¹ The readings at the second (suboptimal) wavelength can be related to those at the first by multiplying by the ratio of the relative absorbances at the two wavelength points. For reference, relative absorbances of SRB in the solubilization reagent are listed in Table 1. Since SRB is fluorescent, data readout can also be obtained from a fluorometric microplate reader. It is necessary to use a suboptimal fluorescence excitation wavelength to obtain a linear relationship between fluorescence intensity and cell number.

Notes

[A] To reduce background, only cellular protein should be included in the measurement. Consequently, it is important to use a serum (protein)-free medium compatible with the cell type of interest.

[B] Any standard microplate may be used; volumes specified here are for the 96-well type.

[C] It is important during and after addition of TCA that the cells be disturbed as little as possible. Jolting of the suspensions may result in nonattachment of cells and a subsequent underestimate of protein quantity.

[D] To apply the assay to adherent cells, reduce the final concentration of TCA added to 10%. Use a stock solution of 50% (w/v) TCA diluted 5-fold in step 3.

[E] Once the plates are stained with SRB, it is recommended they be kept in the dark. If the assay is stopped at any point the samples should be covered (e.g., by another plate) to prevent protein contamination.

References

1. Eur J Cancer 29A, 395 (1993); 2. J Natl Cancer Inst 82, 1113 (1990); 3. J Natl Cancer Inst 82, 1107 (1990).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
F-2961	FluoReporter® Colorimetric Cell Protein Assay Kit	1 kit

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Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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