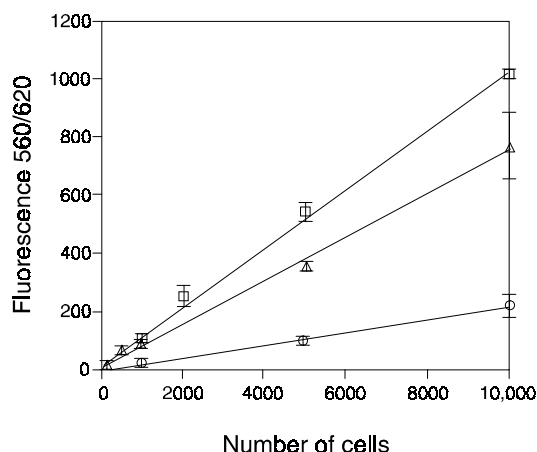


## FluoReporter<sup>®</sup> Fluorometric Cell Protein Assay Kit (F-2960)

### Introduction

This kit implements an electrostatic dye binding assay for cellular protein,<sup>1</sup> adapted for a fluorescence microplate reader.<sup>2</sup> The red fluorescent anionic dye sulforhodamine 101 (SR 101) forms an electrostatically stabilized complex with basic amino acid residues under moderately acid pH conditions. This complex can be detected as a linear increase in dye binding (fluorescence) with protein content after wash removal of unbound dye. A non-fluorescent counterstain is used to prevent saturation of the fluorescence response by reducing the number of available binding sites. The assay is applicable to both adherent and non-adherent cell types (Figure 1). Our development work has included demonstrating the use of this method for monitoring the time course of mitogen stimulated lymphocyte growth.<sup>2</sup> Other potential applications include cell counting, monitoring cell size changes and testing of antibiotics. The protocol has been optimized to measure between 100 and 100,000 cells. The kit should be particularly useful for the analysis of large sample numbers, particularly as plates may be stored at two stages in the protocol allowing the sequential scan of plates from different time points.



**Figure 1.** Comparison of SR 101 staining of three different cell types. P3X (a non-adherent murine myeloma cell line (Δ)), 3T3 (an adherent murine fibroblast cell line (□)) and PBL (goat primary peripheral blood lymphocytes (○)) were stained according to the standard protocol described in *Experimental Protocols*. All cell types showed a linear increase in fluorescence with cell number. In addition, the fluorescence signal magnitudes reflect the relative sizes of the cells. Lymphocytes and P3X cells have diameters of 8 and 15 microns respectively. Detection limits were found to be between 100 and 500 cells depending on cell size.

### Materials

#### Contents

- **100 μM sulforhodamine 101 solution** 20 mL in 1% acetic acid
- **Concentrated counterstain solution** 20 mL in 1% acetic acid
- **Concentrated solubilization solution** 16 mL

#### Storage

The contents are sufficient for 2000 microplate samples following the standard protocol (see *Experimental Protocols*). The reagents should be stored at 4°C until required. The sulforhodamine 101 and counterstain reagents contain 2 mM sodium azide preservative.

#### Materials Required but Not Provided

- Microplates with a capacity of ≥250 μL per well
- Fluorescence microplate reader
- Trichloroacetic acid (see *TCA Fixation Solution*)
- Glacial acetic acid
- Hemacytometer

### Preparation

#### TCA Fixation Solution

The fixing solution is 80% (w/v) trichloroacetic acid (TCA). 100 mL of this reagent is sufficient to fix 2000 microplate wells of non-adherent cell suspension. Adherent cells require lower TCA concentrations (see *Staining with 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 to prepare both the staining solution (see *SR 101 Staining Solution*) and as a wash reagent (step 1.8). To prepare 1% acetic acid, slowly add 10 mL of glacial acetic acid to 990 mL of distilled water. Several (5–7) liters of this reagent will typically be needed to run all the samples provided for by this kit, primarily for the washing step.

#### SR 101 Staining Solution

The staining solution consists of a mixture of sulforhodamine 101 and nonfluorescent counterstain in 1% acetic acid. To prepare the staining solution, mix 1 mL of the 100 μM sulforhodamine 101 solution (see *Contents*) and 1 mL of the concentrated

counterstain and then dilute with 8 mL of 1% acetic acid (see *1% Acetic Acid Solution*). The resulting 10 mL of staining solution is sufficient to treat 100 microplate samples (~1 × 96 well microplate) according to the standard protocol.

This reagent may be stored in the dark at room temperature if not required immediately.

### **Solubilization Reagent**

Add the entire supplied amount (16 mL) of concentrated solubilization reagent (see *Contents*) to 400 mL of distilled water. The resulting volume of solution (416 mL) is sufficient to treat all the samples provided for by this kit (approximately 2000). Store the dilute solubilization reagent in a refrigerator.

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## **Experimental Protocols**

### **Construction of a Standard Curve**

To construct a standard curve for a particular cell type, follow the standard protocol (see steps 1.1–2.2) using serial dilutions of a suspension of known cell number density determined with a hemacytometer. This is particularly important for proliferation studies.

### **Staining Non-Adherent Cells**

**1.1** Wash cells in serum-free medium [note **A**] and resuspend to a concentration of ~10<sup>6</sup> cells/mL.

**1.2** Serially dilute cells into microplate wells [note **B**] to densities in the range of 500 to 100,000 cells in 200 µL. 200 µL control samples of cell-free medium are recommended for fluorescence blank readings (step 2.2).

**1.3** Gently layer 50 µL of 80% w/v trichloroacetic acid [note **C**] onto each 200 µL cell sample (final concentration of 16% [note **D**]).

**1.4** Store plates in the refrigerator for 1 hour.

**1.5** 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.

**1.6** Dry plates in air (if overnight storage is desired), or in an oven for about 30 minutes at 45–50°C.

**1.7** Add enough SR 101 staining solution (see *SR 101 Staining Solution*) to completely cover the bottom of the wells (100 µL per well is usually sufficient for a standard 96-well plate) and incubate for 30 minutes in the dark at room temperature.

**1.8** Repeat wash step (step 1.5) using 1% acetic acid in place of tap water.

**1.9** Air- or oven-dry the samples as described above (step 1.6); plates may also be stored at this point [note **E**].

**1.10** Prepare the plate for reading by addition of 200 µL diluted solubilization reagent (see *Solubilization Reagent*) followed by gentle agitation for a few minutes to ensure complete dissolution.

**1.11** Proceed to *Fluorometric Readout*.

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## **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 non-attachment 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 fivefold in step 1.3.

[**E**] Once the plates are stained with SR 101, 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.

### **Fluorometric Readout**

**2.1** Measure fluorescence using a microplate reader with excitation and emission filters centered at 560 nm and 620 nm, respectively. Alternative filter sets compatible with the excitation and emission spectra of SR 101 may also be used. The fluorescence excitation and emission maxima of SR 101 in the solubilization reagent are 586 nm and 602 nm, respectively. The instrument detection sensitivity should be adjusted to give on-scale signals for all samples.

**2.2** Subtract blank fluorescence values from sample data before plotting or other subsequent analysis.

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## **References**

1. J Natl Cancer Inst 82, 1107 (1990); 2. J Cell Biol 115, 83a (1991).

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