Gene Therapy Research Reagent

CellScrub™ Buffer

Washing Buffer

Product Summary

Cat. No:	B100001
Description:	100 ml of CellScrub™ Buffer
Storage:	Store at 4°C.
Comments:	Suitable for use in gene delivery studies. GenePORTER (Optimized transfection reagents) and pGeneGrip™ (fluorescein, rhodamine or biotin labeled plasmid DNA) from Gene Therapy Systems are sold separately.

INTRODUCTION

CellScrub™ Buffer is a unique washing buffer designed to remove all cationic lipid/DNA complexes associated with cell surfaces. The CellScrub™ Buffer is non toxic for the cells and allows discrimination between extracellular and intracellular plasmid DNA. The use of the CellScrub™ Buffer as part of a cell washing procedure allows more precise quantification of the amount of DNA delivered into the cells for DNA stability or cytometry studies.

USAGE

The CellScrub Buffer is stored at 4°C and can be left at room temperature before use.

Include the CellScrub Buffer in your regular washing procedure (see example protocols).

RELATED PRODUCTS

pGeneGrip™ products are biologically active plasmid DNA irreversibly labeled with Fluorescein, Rhodamine or Biotin. Please contact us for a complete list of pGeneGrip™ plasmid products bearing different labels and various encoded reporter gene.

Gene Therapy Systems, Inc.
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Why CellScrub™ (washing) buffer?

The quantification of DNA delivered by cationic lipids into cells and the analysis of the intracellular DNA stability represents a topic of major interest in non-viral gene delivery research. But determining intracellular DNA requires a washing procedure to remove all of the plasmid that is attached on the cell surface while keeping the cells alive. We have found that trypsinization and washing with PBS is not sufficient to remove cationic lipid/DNA complexes associated with the cell surface (see gel lane 1). We therefore developed a washing buffer (**CellScrub™**) that allows removal of all the extracellular plasmid (see gel lane 2) without altering cell viability. This washing buffer should be a useful tool to help differentiate between the amount of DNA bound to the cell surface from the DNA that is internalized into cells. This buffer allows more quantitative analysis of DNA delivery to be more accurately determined.

Advantages of the CellScrub™ buffer:

- Ready and easy to use
- Cell viability intact
- Removes all extracellular DNA associated with cationic lipids

Example Protocols:

DNA stability study

Adherent cells cultured in 6-well plates are transfected with 5µg of DNA complexed with cationic lipids per well as described *. After the desired times of incubation, culture medium is removed and cells are washed once with PBS (containing calcium chloride and magnesium chloride). Cells are then treated at room temperature for 10-15 min. with the **CellScrub™** buffer (1-2 ml / well) to remove all extracellular cationic lipid/DNA complexes. After two more washes with PBS (without calcium chloride and magnesium chloride), cells are trypsinized, washed once again with PBS, centrifuged and lysed. Finally, the DNA extraction, agarose gel electrophoresis and southern blotting can be done according to standard procedures.

*Felgner, J.H. *et al.* Cationic lipid-mediated transfection in mammalian cells: "Lipofection". *J. Tiss. Cult. Meth.* **15**, 63-68 (1993).

Quantitative flow cytometry analysis of DNA delivered by cationic lipid

Intracellular plasmid delivery and transgene expression can be measured simultaneously by flow cytometry using the **pGeneGrip™ vector** (for example, Rhodamine/GFP; Cat # G101045 where rhodamine is the intracellular DNA marker and green fluorescent protein the transgene expression marker). Cell transfection, fixation and flow cytometry analysis can be done according to Tseng *et al.* **
In order to insure that the fluorescence is derived from intracellular plasmid, the cell washing procedure described above for the DNA stability study must be applied.

Tseng, W.-C. *et al.* Transfection by cationic liposomes using simultaneous single cell measurements of plasmid delivery and transgene expression. *J. Biol. Chem.* **272, 25641-25647 (1997).

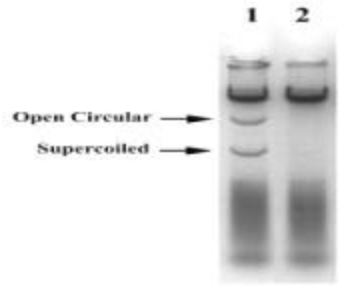

Figure 1. Removal of extracellular cationic lipid/DNA complexes analyzed by agarose gel.

Adherent cells were incubated with cationic lipid/DNA complexes at 4°C for 1-2 hours. Then, several washing procedures were used to remove cell-associated complexes. After washes, cells were lysed, the DNA was extracted and analyzed by agarose gel electrophoresis. DNA was stained with ethidium bromide. Arrows show plasmid DNA. **Lane 1:** Trypsinisation and PBS washes. **Lane 2:** Washing procedure including the washing buffer.

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