CellScrub™

Washing Buffer

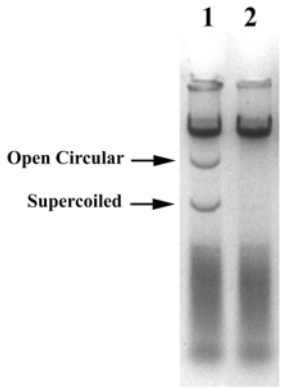
Cat. #	Contents	Quantity
B100001	CellScrub Washing Buffer	1 x 100 ml

Shipping	Shipped on blue ice
Storage	Store CellScrub Washing Buffer at 4°C.

Related Products	Catalog #
GenePORTER® Transfection Reagent, 75 rxns.	T201007
GenePORTER® 2 Transfection Reagent, 75 rxns.	T202007
GeneSilencer® siRNA Transfection Reagent, 200 rxns.	T55500750
BioPORTER® Protein Delivery Reagent, 24 rxns.	BP502401
Cytofectin™ Transfection Reagent, 1 ml	T610001
Perfectin™ Transfection Reagent, 100 rxns.	T303007
BoosterExpress Reagent, 3 x 1.5 ml	T20100B

Introduction: CellScrub Buffer is a unique washing reagent designed to remove all cationic lipid/DNA complexes associated with cell surfaces after transfection. The CellScrub Buffer is non-toxic for the cells and allows discrimination between extracellular and intracellular plasmid DNA (see Figure 1 on the right. 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.

Figure 1. Removal of Extracellular Cationic lipid/DNA Complexes By the CellScrub Washing Buffer.
Adherent cells were incubated with cationic lipid/DNA complexes at 4°C for 1-2 hours. 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 CellScrub Washing Buffer.



METHODS AND PROCEDURES

A. Example Protocol: DNA Stability Study

1. Transfect adherent cells cultured in 6-well plates with 5μg of DNA complexed with cationic lipids per well as described*.
2. After the desired times of incubation, remove culture medium and wash cells once with PBS containing calcium chloride and magnesium chloride.
3. Treat the cells at room temperature for 10-15 minutes with the CellScrub Washing Buffer (1-2 ml/well) to remove all extracellular cationic lipid/DNA complexes.
4. After two more washes with PBS (without calcium chloride and magnesium chloride), trypsinize the cells, then wash them once again with PBS.
5. Centrifuge and lyse cells as desired. Perform DNA extractions, agarose gel electrophoresis, or Southern blot analysis according to standard procedures.

NOTE: 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.

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

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).

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