ProScience Inc.

STA-PUT

Velocity Sedimentation Cell Separator

for Rapid, Easy Cell Separation

Developed by Johns Scientific
in co-operation with
THE ONTARIO CANCER INSTITUTE, Toronto, Canada

ProScience Inc. - Glass Shop Division
770 Birchmount Road, Unit #25, Scarborough, Ontario M1K 5H3 Canada
Phone: (416) 699-5555 • Fax: (416) 751-4224

THE STA-PUT APPARATUS

The 'STA-PUT' apparatus, designed by Doctors R.G. Miller and R.A. Phillips of the Ontario Cancer Institute in Toronto, permits rapid, easy separation of suspensions of living cells on the basis of differences in cell sedimentation rate in the earth's gravitational field. Under the conditions of the separation, cells sediment at a rate determined primarily by their size, although density also makes a minor contribution. Separation on the basis of size often yields a separation on the basis of function so that separations of functionally different cells can often be achieved.

In a typical experiment, using bovine serum albumin (BSA) as a gradient material to stabilize against convection, the procedure might be as follows: The cells suspended in 0.2% BSA in Phosphate Buffered Saline (PBS) are loaded through the bottom of the sedimentation chamber. This is a cylindrical tank, 12 to 45 cm in diameter, with a conical bottom having a hole at the apex of the cone. Next, a nonlinear gradient of 0.3% - 2% BSA in PBS is introduced slowly under the cell suspension. This lifts the cells to form a thin band near the top of the chamber. The cells are allowed to sediment under the action of gravity for 3 to 14 hours at 4°C, and fractions, usually about 30 in number, are collected through the bottom.

The method has the advantages of being set up and performed easily, and is highly reproducible. A wide range of gradient materials has proven satisfactory, including bovine serum albumin, fetal calf serum, and Ficoll. The gradient is present only to prevent convection; at no point is it sufficiently dense for any cells to approach their equilibrium density. Unlike the conditions often encountered in such alternative methods as density separation, the forces and gradient materials used have almost no effect on the cells. In particular, there is almost no effect on their subsequent viability.

The 'STA-PUT' apparatus was designed in close co-operation with Dr. Miller, with complete attention to minute details. The present design minimizes the problems associated with cell loading, turbulence, cell loss and so forth. Researchers using this apparatus for the first time can normally be assured of results consistent with those described in the literature on their first trial.

REFERENCES

1. Miller, R-G., In Vitro Methods in Cell Mediated and Tumor Immunity, 283-307, edited by Bloom, B.R., and David, J.R., Academic Press (1976) Separation of Cells by Velocity Sedimentation.
2. Miller, R.G., and Phillips, R.A., Proc. Soc. Exp. Biol. Med. 135, 63-67, (1970) Sedimentation Analysis of the Cells in Mice Required to Initiate an in vitro Immune Response to Sheep Erythrocytes.
3. Phillips, R.A., and Miller, R.G., J. Immunal. 105, 1168-1174, (1970) Physical Separation of Hemopoitic Stem Cells from Cells causing Graft-vs-Host disease.
4. Lam, D.M.K., Furrer, R., and Bruce, W.R., Proc, Nat. Acad. Sci. (U.S.) 65, 192-199 (1970) The Separation, Physical Characterization, and Differentiation Kinetics of Spermatogonial Cells of the Mouse.
5. Miller, R.G., and Phillips, R.A., J. Cell Physiol. 73, 191-201, (1969) Separation of Cells by Velocity Sedimentation.
6. Worton, R.G., McCulloch, E.A., and Till, J.E., J. Cell. Physiol. 74, 171- , (1969) Physical Separation of Hemopoietic Stem Cells from Cells Forming Colonies in Culture.

STAPUT FEATURES WORTH CONSIDERING ....

1. SYSTEMS ENGINEERED - all parts interchangeable and internally compatible.
2. THREE SIZES - cell chambers of 120, 180, and 250 mm diameter, all use same gradient former components.
3. SPECIAL GRADIENT FORMER - volume of gradient chambers in proper proportion to cell loading factor for all liquid levels.
4. DURABLE CONSTRUCTION - materials carefully selected for maximum corrosion-resistance in a laboratory cold room; all parts stainless steel, aluminium, glass, silicone rubber, or teflon.
5. AUTOCLAVABLE - Pyrex glass chambers and silicone rubber parts can be steam sterilized repeatedly for aseptic experiments.
6. LOW CELL LOSS - materials and surfaces carefully selected to minimize cell loss; typical recovery is 95% or more.
7. CELL CHAMBER - visible for easy observation of sedimentation bands. Level indicator allows exact reproduction of chamber volume.

APPLICATION

Consider cells having volume of about 100μ^3. Then, with buffered step gradient, $\sim 10^7$ cells/mL can be loaded before streaming occurs (ref.). For best resolution the starting band should have a band $\sim$ 2.5 mm. wide. Therefore, maximum cell loads are:

56700-012 (10mL/mm) $\sim$ 2.5 x 10^8 cells.
56700-001 (25mL/mm) $\sim$ 6.3 x 10^8 cells.
56700-019 (50mL/mm) $\sim$ 12 x 10^8 cells.

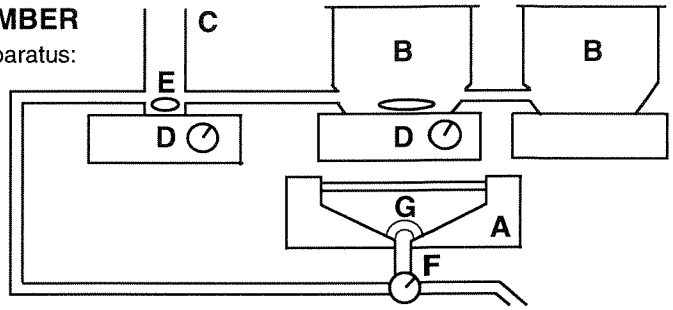
To a 1st approx., the streaming limit is inversely proportional to cell volume. Thus, for example, only 2.5 X 10^7 cells of volume 1000μ^3 can be loaded in a 56700-012 chamber.

PROCEDURE FOR CELL SEPARATION BY VELOCITY SEDIMENTATION MATERIALS

1. Stock solutions of 20% bovine serum albumin in Dulbecco's phosphate buffered saline (PBS) and additional PBS for dilutions. Both should be filtered if electronic cell counts are to be done. Other gradient intervals (eg. Ficoll, serum), diluted to give the same density range as the BSA gradient described below are also satisfactory. Low molecular weight gradient materials, such as sucrose, are not suitable.
2. Cells. Wash by centrifugation at force less than 250 x g. Resuspend in 0.3% BSA in PBS.
3. Cold room or vibration-free fridge. The separation is best done at 4°C. All materials and equipment should be kept at this temperature.

SETTING UP THE CHAMBER

Assembly of the STA-PUT apparatus:



A - sedimentation chamber (56700-500)
B - graduated gradient chambers (56700-300)
C - cell buffer chamber (56700-400)
D - magnetic stirrers (56700-200)
E - teflon spin bars for
cell buffer chamber (58948-219)
first gradient chamber (56700-110)

F - 3-way micro metering valve (56700-800)
G - STA-PUT baffle (56700-700)

All tubing through which cells pass should be silicone (25390-410).

LOADING AND UNLOADING THE CHAMBER (56700-001)

(The quantities given below are representative only, and should be varied according to the sedimentation time and resolution desired. When using a different chamber, quantities can be scaled roughly as the ratio of the surface areas.)

1. Fill all connecting tubing with PBS, making sure it is free of air bubbles.
2. Center STA-PUT baffle inside cone of sedimentation chamber. (Center carefully - this is important.)
3. Clamp lines between all three gradient chambers. Load 600 mL 1% BSA into left hand bottle of (B) and 600 mL 2% BSA into right hand bottle.
4. Load top layer (50 mL PBS) into chamber through the cell buffer chamber (C). Check that STA-PUT baffle is still correctly centered.
5. Load cells in 40 mL of 0.3% BSA in PBS, through buffer chamber (C). The cell concentration must be below the streaming limit for the cells being loaded. For sheep erythrocytes (volume of 31u^3) 1.5 x 107 cells/mL is satisfactory. To make estimates for other cells, scale this value inversely with the cell volume. While cells are loading, adjust needle valve for flow rate of about 2 mL/minute. Clamp line the instant buffer chamber empties.
6. Rinse buffer chamber twice with PBS. Use a 50 mL syringe and a piece of tubing to do this. Check that no air bubbles entered the line during the rinse.
7. Fill (C) with buffer gradient (0.5% BSA in PBS) to level of fluid in gradient bottles. Check that needle valve is at 2 mL/min. rate. Turn on magnetic stirrers. Remove all clamps and the gradient will load itself.
8. Once the cells have been lifted off the bottom, the flow rate can be increased. Continuously adjust by eye to rate just below that at which cell band is distributed. Small disturbances will settle out.
9. After appropriate sedimentation time (usually 3-4 hours) unload chamber through bottom at a rate of about 60 mL/min. Discard cone volume (approx. 350 mL). Thirty mL is a convenient fraction size.