

Stem cells PSC, Pluripotent Stem Cells.
PSC-derived functional cell types (e.g. cardiomyocytes).
UniFuge with 800 ml Shallow pool module.
Viable aggregate and dissociated Cell collection,
buffer washes, media exchange.

David Richardson, Pneumatic Scale Angelus
david.richardson@bwpackagingsystems.com

5320 140th Avenue North
Clearwater, FL 33760 USA
t. +1(727) 535-4100
f. +1(727) 539-8944
psangelus.com

Aggregated Stem cells were grown in a Eppendorf 5 L SU Bioreactor.
Purpose is to concentrate/collect stem cells aggregates and wash cell
aggregates. Remove cells from Single use module. Treat cell aggregates with
disassociation buffer then wash/collect dissociated cells with UniFuge.
Expectation: close process with 90% or greater recovery and viability.

Experiment:

PSC stem cells are grown in cell clusters called aggregates.
The Centrifuge Equipment is a UniFuge with 0.8 L shallow pool Module.
Cell health 96.6 % viability. 0.7 E5, 3.7-liter feed
Process parameters are:
G force 700 x g. Feed flow rate .75 lpm.
Pre-fill bowl with 800 ml PBS buffer.
Separation 6 minutes, then wash with 1.0-liter PBS 0.5 l/m.
Stop centrifuge to collapse separation zone.
Continue cycle and wash with 1.0-liter PBS by continuing cycle, then
remove cells with 3- 0 ml e-suspensions: 300 ml, 300 ml, 200ml volumes.

Result: 94 % viability. 100 % recovery.

Part 2:

Treat collected PSC aggregates 0.8 l with dissociation buffer 3.2 litres.
Process 4.0-liter feed
Process parameters are:
G force 700 x g. Feed flow rate .75 lpm.
Pre-fill bowl with 800 ml PBS buffer.
Separation 4.0 liters feed.
Wash with 1.0-liter PBS 0.5 l/m.
Stop centrifuge to collapse separation zone.
Continue cycle and wash with 1.0-liter PBS by continuing cycle.
Remove cells with 3- 0 ml resuspensions. 300 ml, 300 ml, 200ml volumes.

Results: 0% loss in centrate. 90 % viability. 100 % recovery.

Discussion:

We optimised process by removing dissociation step from module. Also, by
collapsing separation zone and washing cells with 2- 1-liter pbs wash steps.
We met expectation of customer.

