



50mL TubeSpin Protocol

Materials:

Complete Cell Media (CD-FortiCHO + 1:1000 ACA)

TubeSpin® Bioreactor 50 (TPP, Cat#87050)

10uL, 200uL, and 1mL Filter Tips

Pipettes

Serological Pipettes

Pipette Controller Cell

Counting Slides Trypan

Blue (filtered)

Media Booster: 7A (Cytiva, Cat# SH31119.02); 7B (Cytiva, Cat# SH31120.03)

15mL and 50mL Falcon tubes

1.5mL reaction tubes

Glucose monitor

Osmolarity Meter

Prior to beginning:

- 1) Thaw cells into E125 (0.3×10^6 cells/mL, total 30mL) and recover for one week.
- 2) Prepare cells: 1×10^6 cells/ml in 15ml (15×10^6 cells/Tube) of a Spin tube
 - a. Example: 2 tubes, prepared 35×10^6 cells (extra 5×10^6 cells) into 50mL tube
 - b. Spin down cells at 125xg for 7 minutes
 - c. Remove supernatant and add 31ml warmed media (dilute always easier way)
 - d. Recount cells, acceptable range is $0.95-1.1 \times 10^6$ cells/mL
- 3) Seed the cell into Spin tube (1×10^6 cells/mL, 15mL, total 15×10^6 cells each tube)
- 4) Place the cells on a shaker inside the incubator: 240rpm, 37°C, 8% CO₂
- 5) Once cells are in the incubator, prepare Cell Boost Feeding Media for duration of the 14-Day experiment
 - a. Calculate the amount of feeding media needed to feed all wells for duration of experiment (add some more for comfort)
 - b. Vacuum filter the appropriate amount of Cell Boost 7A and Cell Boost 7B into SEPARATE 50mL tubes. Store @ 4°C
- 6) Start the daily Cell Boost feeding schedule on Day 3
 - a. 7a and 7b are separately warm for 5 minutes in 37°C water bath.
 - b. Add the feeding media at 2% of 7A: 300uL and 0.2% of 7B: 30uL per well
 - c. Continue feeding once per day, including Day 13. No feed on the final Day 14.
- 7) On Day 4, turn the temperature down to 32°C
 - a. While the incubator is lowering the temperature, feed the cells.
- 8) Glucose Measurements (Start on Day 3):
 - a. measurements should be between 3-4 g/L.
 - b. Test Glucose level on Day 3, Day 5, Day 7 and after (everyday)
 - c. If the glucose level is < 3g/L: add stock glucose (200g/L) to bring the concentration to 4g/L. And then feed with booster media to 2% (e.g. add 300uL of 7A and 30uL of 7B to 15ml).
 - d. If the glucose level is 3g/L – 5g/L: Do not add glucose. Feed with booster media to 2%



(e.g. add 300uL of 7A and 30uL of 7B to 15ml).

- e. If the glucose level is **>5g/L**: Do not add glucose. Do not feed with booster media.

- 9) On Day 7, Day 9 (or Day 10), Day 12 (or Day 11), and Day 14: count cells (may need to perform a dilution prior to mixing with trypan blue), collect supernatant.
 - a. Take viability and cell density (take 10uL to counted VCD directly).
 - b. Spin the entire falcon tube @ 125xg for 7 minutes
 - c. Take 300uL supernatant for measuring glucose concentration and osmolarity, leftover-> Valita assay (Titer test) (on D14 or after) and the samples @ -80°C.
 - d. Feed and resuspend the cell

DAY	Action
DAY 3	Begin feed, add 300uL of 7A and 30uL of 7B feed media each well, 37°C
DAY 4	Add 300uL of 7A and 30uL of 7B feed media each well, Switch temperature to 32°C
DAY 5	Add 300uL of 7A and 30uL of 7B feed media each well, 32°C
DAY 6	Add 300uL of 7A and 30uL of 7B feed media each well, 32°C
DAY 7	Count cells, collect 300uL supernatant and test Osmolarity and Glucose level leftover->for Valita store at -80. Add 300uL of 7A and 30uL of 7B feed media each well, 32°C
DAY 8	Add 300uL of 7A and 30uL of 7B feed media each well, 32°C
DAY 9	Count cells, collect 300uL supernatant and test Osmolarity and Glucose level leftover->for Valita store at -80. Add 300uL of 7A and 30uL of 7B feed media each well, 32°C
DAY 10	Add 300uL of 7A and 30uL of 7B feed media each well, 32°C
DAY 11	Add 300uL of 7A and 30uL of 7B B feed media each well, 32°C
DAY 12	Count cells, collect 300uL supernatant and test Osmolarity and Glucose level leftover->for Valita store at -80. Add 300uL of 7A and 30uL of 7B feed media each well, 32°C
DAY 13	Add 300uL of 7A and 30uL of 7B feed media each well, 32°C
DAY 14	Count cells, collect 300uL supernatant and test Osmolarity and Glucose level leftover->for Valita store at -80.

Glucose Measurements (measure this once you have supernatant aliquots ready for all samples)

- a. Insert strip into monitor
- b. Add 1.5uL into the side of the capillary
- c. Measurement should take 3-5 seconds per sample, and most measurements should be between 3-4 g/L

Osmolarity (OSM) (measure after feeding the cells, basically last task to do)

- a. Warm up the machine for 10 minutes (do not warm for longer as machine will turn
- b. Run the standards three times (three separate tubes with 30uL in each tube) NO AIR BUBBLES!

Note* Between each sample/tube, use a kim wipe to wipe off probe.

- c. Once the standard looks consistent over three measurements, proceed with samples.
 - a. Samples → 30uL of the supernatant per tube, measure twice, if numbers aren't within 5-10 units of each other, process a third sample.

