Protocol for freezing aliquots of SH-SY5Y cells

You will need: fetal bovine serum (FBS), 0.25% trypsin EDTA, SH-SY5Y media (DMEM:F12, 10% FBS, 1% pen/strep), sterile DMSO, cryovials

1. To prepare to freeze down aliquots of cells, passage cells into as many 75cm^2 flasks as can be made, adding 1 million cells per flask.
2. When cells are ~60% confluent, prepare to freeze aliquots by warming FBS, 0.25% trypsin EDTA, and SH-SY5Y media.
3. When FBS, trypsin, and media are warm, remove existing media from flasks and add 2 mL of trypsin to each flask.
4. Place in the incubator for 5 minutes.
5. Add 6 mL of fresh media to each flask, washing the bottom of the flask to ensure all cells have been detached. Add suspended cells into a 15 mL conical tube. Repeat for all flasks.
6. Centrifuge 15 mL conical tubes containing suspended cells for 3 minutes or until a cell pellet is visible.
7. Remove media, leaving the cell pellet undisturbed.
8. Add 1 mL of FBS to each 15 mL conical tube containing a cell pellet. Resuspend the pellet and transfer all suspended cells to a single conical tube.
9. Count the cells. Dilute with FBS to a count of 4 million cells per 1 mL.
10. Prepare a 20% solution of DMSO in FBS of an equal volume to the cell suspension.
11. Prepare cryovials with correct labels and remove caps.
12. Add 20% DMSO in FBS solution to cell solution and immediate begin filling cryovials with 1 mL of solution (2 million cells, 10% DMSO in FBS).
13. Work QUICKLY to fill all cryovials and re-cap them. Transfer them immediately to a -80° C freezer.
14. Allow cryovials to remain in the -80° C freezer for 48 hours before transferring to them to a liquid nitrogen cell storage dewar.