UCH Chordoma Cell Lines Culturing Instructions

IMPORTANT: YOU SHOULD FREEZE DOWN SEVERAL VIALS AS CELLS STOCK AS SOON AS YOU HAVE SUFFICIENT CELLS. TEST THE FREEZING TO ENSURE VIABLE CELL RECOVERY AND CONTINUED GROWTH BEFORE STOPPING CULTIVATION OF THE ORIGINAL CULTURE(S).

Thawing: To thaw frozen cells, quick thaw vial by warming in 37^{o}C water bath. Dilute cells in 10 mls of pre-warmed complete media. Centrifuge cells 10 minutes at 250xG. Based upon the number of cells frozen, resuspend cells in appropriate volume and replate on gelatin-coated plates (for vial containing equivalent of 100 mm plate, I resuspend in 12-15 mls for replating and replate back into 100mm dish).

Passing Cells (UCH cells should be grown in 5% CO_2 in humidified incubators)

. 1) Coat plates or flasks with sterile 0.1% gelatin solution (dilute 2% solution (Sigma cat # G1393) 1:20 in water, remaining unused 0.1% solution may be kept for later usage- store at 4oC) - prewarm the gelatin solution in 37^{o}C bath, then add to plates/flasks (typically I use 2.5-3 mls per T75 or 100mm, be sure it covers surface during incubation), incubate at 37^{o}C. After 20 minutes remove the gelatin solution, lean plates/flasks to allow drainage and leave for 20+ minutes to dry in sterile tissue culture hood. At end of the drying period, aspirate remaining solution and use to replate cells. Alternatively a large batch of flasks/plates may be precoated then used later if stored in sterile conditions at 4^{o}C (rebag in same sleeve)

. 2) Remove cells from incubator, aspirate off old media, and wash with appropriate volume of PBS

. 3) Add appropriate amount of RT or warm trypsin/EDTA solution (Invitrogen/GIBCO 25200-056) (1 ml trypsin solution /100 mm plate).

. 4) Gently rock/swirl to cover cells then remove 50-75% of the trypsin solution (leave sufficient to cover surface).

. 5) Return to CO_2 incubator and examine every 1-2 minutes until cells are loosely adherent- if you use 0.05% trypsin this may take several minutes. For 0.25% trypsin containing solutions, it will be much quicker and cells should be watched closely. Remove from incubator and shake/tap the flask vigorously to dislodge cells from substrate.

. 6) Add complete media (see below for growth media) and split cells into 2 or 3 new flasks or plates of similar size. Split 1:2 or 1:3

. 7) Return to incubator and refeed twice a week until ready to split again.

NOTE: Do not allow the cells to grow to high densities or confluence for any extended period. These conditions will enrich for a subpopulation of cells that are not representative of chordoma cell lines.

Freezing: To freeze cells, trypsinize, as above, neutralize trypsin by addition of complete media and pellet cells. Resuspend cells in complete media containing 10% DMSO, working quickly aliquot, transfer vials into appropriate slow freeze apparatus (Nalgene Cryo-freezer container Cat # 5100-0001) and move into -80^{o}C freezer. The next day, transfer to LN_2 for long-term storage.

Freeze 1 x 100 mm plate (or equivalent) in 1 ml freezing media per

vial as this will allow quick expansion following thawing.

Notes:

Cell lines grow slowly with a doubling time is approximately 5 days. Change media twice per week and split cells when ~90% confluence. UCH lines contain physaliferous (bubbly) cells containing various numbers and sizes of vacuoles, however not all cells are physaliferous as this varies from line to line. Unlike some other cell lines, the presence of vacuoles is a sign of optimal growth, rather than stress.

Media:

IMDM (Invitrogen 12440)/RPMI 1640(Sigma R8758) four to one ratio - (4 IMDM:1 RPMI) 10% FBS (Invitrogen/GIBCO 16000-044).

Media preparation: 400 ml IMDM, 100ml RPMI, 50ml FBS, 5ml pen-strep

**For questions email Patty Cogswell (patty@chordoma.org)

These cell lines were created by Silke Bruederlein