FACLR
FRIEDREICH'S ATAXIA
CELL LINE REPOSITORY

Napierala Lab protocol for human skin fibroblast culture (May 2022)

Necessary Reagents

Reagent	Company	Catalog Number
DMEM, high glucose	Gibco/Life Technologies	11965-092
Fetal bovine serum	Hyclone	SH30910.03
MEM Non-essential amino acids (NEAA)	Gibco/Life Technologies	11140-050
0.25% Trypsin-EDTA, phenol red	Gibco/Life Technologies	25200-056
DPBS (1X)	Gibco/Life Technologies	14190-144

Fibroblast Medium recipe:

DMEM, high glucose

15% fetal bovine serum (FBS)

1% MEM Non-essential amino acids (NEAA)

Thawing cells

1.) Preparation: warm Fibroblast Medium in a 37°C waterbath and add 5 mL to new 15 mL conical tubes (1 per cell line)

2.) Take vials from liquid nitrogen dewar

3.) Rapidly thaw vials in a 37°C waterbath until just a small piece of ice remains (< 5 min)

4.) Using aseptic technique, immediately transfer the cell suspension from the vial (1-2 mL) into the 15 mL conical tube containing 5 mL of pre-warmed Fibroblast Medium

5.) Spin the cells at 200 x g for 5 min at room temperature

6.) Aspirate medium without disturbing pellet and flick the bottom of the tube with your finger gently to dislodge the cells

7.) Resuspend cells in 8 mL Fibroblast Medium and transfer to a new, labeled 10 cm plate

8.) Add another 2 mL of Fibroblast Medium to the 15 mL conical tube, rinse, and transfer to the same 10 cm plate

9.) Gently rock the plate from side to side several times to disperse the cells evenly across the bottom

10.) Place in an incubator (37°C, 5% CO_2) overnight

11.) The following day, aspirate medium from each plate and replace with 10 mL fresh Fibroblast Medium (medium should be warmed to 37°C)

12.) Fibroblast Medium will need to be changed every 3 days until cells reach confluence

Splitting/passaging cells (general)

1.) Warm the Fibroblast Medium, trypsin, and PBS in a 37°C waterbath

2.) Aspirate medium from plates

3.) Add 3 mL warm PBS to the plate, and gently rock from side to side to rinse the cells, then aspirate

4.) Add 2 mL of trypsin and place plate into a 37°C incubator for 3-5 min

5.) Prepare new 10 cm plates by labeling and adding 8 mL Fibroblast Medium** to each

6.) Take the cells from the incubator and gently tap the sides of the plate to dislodge the cells

7.) Add 8 mL Fibroblast Medium and pipet up and down to disperse cells into solution (no visible clumps should remain)

8.) Pipet 2 mL of the cell suspension** into each new 10 cm plate

**NOTE: the above ratio (1:5 split) is for routine culture and can be modified to suit experimental purposes. Generally, 1x10^6 fibroblasts/10 cm plate is sufficient for propagation/expansion and will take approximately 7 days to reach confluence.

9.) Gently rock the plate from side to side several times to disperse the cells evenly across the bottom

10.) Place in an incubator (37°C, 5% CO_2) and check cells the following day to verify attachment