FACLR
FRIEDREICH'S ATAXIA
CELL LINE REPOSITORY

Napierala Lab protocol for human skin fibroblast culture

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
Dimethyl sulfoxide (DMSO)	Sigma	D8418-100ML

Fibroblast Medium recipe:

DMEM, high glucose
15% fetal bovine serum (FBS)
1% MEM Non-essential amino acids (NEAA)

Fibroblast Freezing Medium recipe:

40% Fibroblast Medium (culturing medium)
50% fetal bovine serum (FBS)
10% DMSO

NOTE: We culture fibroblasts without the use of antibiotics (e.g. Pen/Strep). Growth medium and freezing medium are prepared and then filter-sterilized (0.22 μm) prior to use.

Thawing cells

1.) Preparation: warm Fibroblast Medium in a 37°C water bath, then add 5 mL to new, labeled 15 mL conical tubes (1 tube per cell line).
2.) Take vials from liquid nitrogen dewar.
3.) Rapidly thaw vials in a 37°C water bath 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 gently flick the bottom of the tube with your finger 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 using front-to-back, side-to-side motions several times to disperse the cells evenly across the bottom. Avoid swirling the plate.
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)

NOTE: The splitting ratio (1:5 split) described below 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 6-7 days to reach confluence.

1.) Warm the Fibroblast Medium, trypsin, and PBS in a 37°C water bath.
2.) Aspirate medium from plates.
3.) Add 3 mL warm PBS to each 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 warm 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).

    NOTE: Early passage fibroblasts (p2, p3) could have been cryopreserved with small tissue pieces remaining from the original skin biopsy. These pieces will not disperse by pipetting. They are not harmful to the culture can be eliminated by aspiration during routine medium changes after the fibroblasts have attached to the plate.
8.) Pipet 2 mL of the cell suspension into each new 10 cm plate.
9.) Gently rock the plate using front-to-back, side-to-side motions 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. There is no need to change the medium the day after routine passaging. Instead, the medium can be changed ~ every 3 days until cells again reach confluence.

Cell freezing (general procedure)

1.) Follow trypsinization protocol (above) through Step 7.
2.) After trypsinizing and collecting in Fibroblast medium, count the cells (preferably using trypan blue exclusion).
3.) Calculate cells needed for the number of cryovials to be prepared and centrifuge the appropriate volume at 300 x g, 3 min, room temperature.

    NOTE: $1x10^6$ cells/cryovial yields good survival upon thawing to a 10 cm dish.
4.) Gently resuspend cells in pre-warmed (37°C) freezing medium at a density of $1x10^6$ cells//mL and aliquot 1 mL of cells per pre-labeled cryovial.
5.) Place the vials in a room temperature Mr. Frosty freezing container and place the container at -80°C for slow cooling (-1°C/min). Keep vials at -80°C for no longer than 1-2 days before transferring to long-term liquid nitrogen storage.