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Human fibroblast culturing

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Fibroblasts are cultured in Dulbecco's modified eagle media (DMEM) 4500 (mg/L) growth medium supplemented with Glutamax (Gibco), 10% foetal bovine serum (FBS), non-essential amino acids (NEAA: 0.1 mM of: glycine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-proline and L-serine) and penicillin/streptomycin antibiotic cocktail (50ng/ml) at 37°C and 5% CO_2.

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SAFETY:-

Biological Hazards:

·The culturing of human cells is a class II activity and all appropriate safety issues need to be addressed.

·If using GMOs read the Laboratory Codes of practice for working with GMOs

Characteristics

1 Characteristics

- Obtained /developed from human adult dermal biopsies

- Proliferative until p20
- Doubling time ~ 24h
- Maintain between 60 – 90% confluent

Complete growth medium

2
- DMEM (LT # 61965-059; 4500 mg/L and no pyruvate) Glutamax medium
- Fetal bovine serum (FBS, 10% final) – 1 / 10 dilution
- Pen / Strep (LT # 15140-122, 50 U/ml and 50 ng/ml final) -- 1 / 200 dilution
- MEM / NEAA (LT # 11140-035) -- 1 / 100 dilution
- As required -- Sodium pyruvate (Sigma # S8636, 1 mM final) – 1 / 100 dilution
- As required -- Uridine (Sigma # U3003, stock of 25 mg/ml in H2O filter-sterilised; 50 ug/ml final) – 1 / 500 dilution
- As required, as permitted by DSO -- Fungizone (LT # 15290-026; original of 250 ug/ml) – 1 / 100 dilution

Subculturing by trypsinisation, and cell counting

3
1. Wash cells grown onto 10 cm Ø plate with PBS.
2. Add 1.5 ml diluted trypsin (4 ml of 2.5%, LT # 15090-046, into 100 ml Versene LT # 15040-033) and leave at 37'C for several mins.
3. Tap the plates and add 2 ml complete medium to stop trypsination.
4. To a 50 ul sample, add equal vol of 0.4% Trypan blue (Sigma # T8154 or LT # 15250-061); fed it to a hemacytometer.
5. Optional -- to pellet cells and resuspend into fresh medium.
6. Replate the cells accordingly; typically 10 ml medium is used for 10 cm Ø plate.
7. Subcultivation ratio of 1:4 is recommended; medium renewal every 2-3d.

Freezing and thawing

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1.After centrifugation to pellet cells, resuspend in Freezing medium (90% FBS / 10% DMSO filter sterilised) and aliquot into freezing vials; typically ¼ of a 50% confluent 10 cm Ø plate of 125K cells in 0.5ml.Freeze cells in a stepwise fashion from -80'C to liq N.
2.Thaw cells at 37'C.

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