IMR-90 growth conditions (ordering info at bottom)

Growth Media (500 ml)

• 500 ml Dulbecco's Modified Eagle's Medium (DMEM)*
• 50 ml 4DN HI FBS (“10%”)
• *No antibiotics*

Culture Conditions

- Atmosphere:
- air, 95%;
- carbon dioxide (CO2), 5%
- Temperature: 37°C

Sub-culturing

Passage IMR-90 cells when they are ~70-90% (*Figure 1*) confluent (~every 3-4 days at a 1:2 – 1:8 dilution). See below for images.

Documentation

Please take pictures from the first 70-90% confluent sub-culture and again when cells are harvested for an experiment. Remember to keep track of doubling since IMR-90’s senesce at 50 – 70 population doublings (normal doubling time ~1day, drops to 1.5 days and longer as cells senesce). The 4DN cells from ATTC will arrive at P14, not sure of exact population doubling level.

Procedure

1. Briefly rinse the cell layer with PBS.
2. Add 2.0 to 3.0 mL of 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to a T-75 flask and place in TC incubator. Check cells every 2 min under an inverted microscope until cell layer is dispersed (usually within 5 minutes).
3. Add 6.0 to 8.0 mL of complete growth medium and mix by gently pipetting.
4. Add appropriate aliquots of the cell suspension to new culture vessels. A subcultivation ratio of 1:2 to 1:8 is recommended
5. Incubate cultures at 37°C.

Medium Renewal: Usually not necessary if passage every 3 to 4 days.

Cryopreservation: Freeze medium: Complete growth medium 95%; DMSO, 5%. Storage temperature: liquid nitrogen vapor phase.

Ordering info:

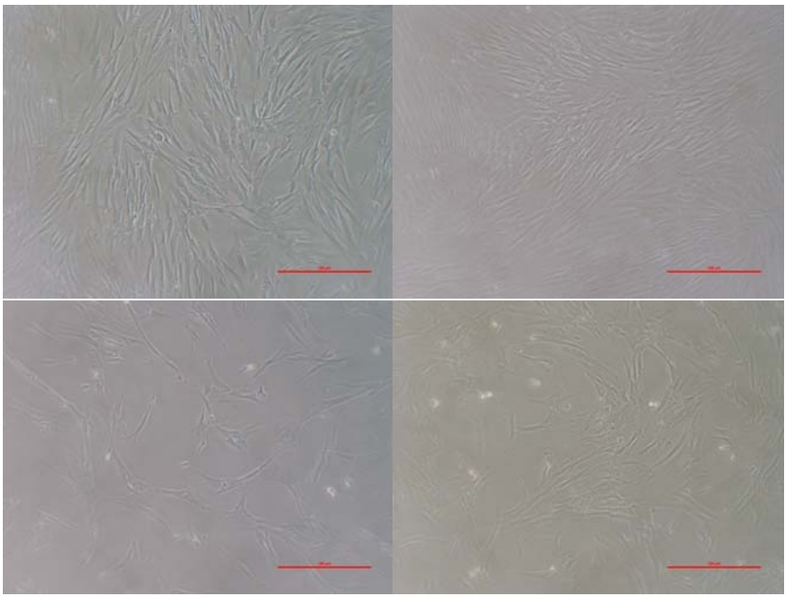
DMEM, Dulbecco’s Modified Eagle Medium 1X
#11965-092 https://www.thermofisher.com/order/catalog/product/11965092

Trypsin-EDTA (0.25%) 1X, phenol red
#25200-056 https://www.thermofisher.com/order/catalog/product/25200056

DPBS 1X (Dulbecco’s Phosphate Buffered Saline)
#14190-144 https://www.thermofisher.com/order/catalog/product/14190144

4DN Seradigm HI FBS: Order from Larry_Stewart@VWR.com, see **ORDERING INFORMATION FOR FETAL BOVINE SERUM (FOR HFF, IMR90 AND GM12878)** on Cell Lines wiki site under Standard Operating Protocols

DMSO: Dimethyl sulfoxide D2650-5X5ML
http://www.sigmaaldrich.com/catalog/product/sigma/d2650



Top row: ready to passage

Bottom row: Not ready to passage

*Recommend DMEM rather than ATCC-recommended EMEM because cells seem to grow slightly flatter in DMEM which is good for imaging.