MCF-7 Cell Culture and +/- estrogen treatment

Source of cells: ECACC (catalog#: 86012803)

DMEM/F12 Red Passage Media

500 ml DMEM Phenol-Red Media, GIBCO/Invitrogen, Cat no: 11330-057 (10 bottles)

25 ml (to 5%) FBS

5 ml Pen Strep

1.5 ml Gentamycin

Filter sterilize

DMEM/F12 Clear Starvation Media

500 ml DMEM Phenol Red Free Media, GIBCO/Invitrogen (with L-Glu), Cat no: 11039-021

25 ml (to 5%) Charcoal Stripped FBS

5 ml Pen Strep

1.5 ml Gentamycin

Filter sterilize

Thawing Cells step:

1. Take one vial out from LN2 tank or -150°C freezer and thaw vial immediately in 37°C water bath. Keep O ring above the water surface to prevent contamination. Thaw content with slight shake until only small ice is left in vial. It usually takes 1 min. Spray vial with 70% ethanol all over and wipe its surface with clean tissue in the hood.
2. Open the vial and transfer the content to a 15 ml Falcon tube. Use 1ml of media (F12+5%FBS+P/S) to rinse the vial and transfer to the 15 ml tube.
3. Add another 5 ml of fresh medium to the 15ml tube slowly. Mix them by pipeting up and down.
4. Spin down at 1000 rpm or 200g for 3-5 mins at 4°C.
5. Resuspend cells in medium and transfer to 75cm2 flask.
5. Check the cells under microscope.
6. Cells are cultured in CO2 incubator and medium is changed about every 3 days.
7. It usually takes 3 days or more for cells to recovery from freezing. After cell culture reachs 80-90% confluence, subculture is conducted. Subculture ratio is about 1:3.

Cell Passage Steps

1. Observe cells to see how confluent they are, whether the cells are alive, whether the cells are contaminated, and whether the cells have the correct morphology.
2. Remove media
3. Wash 1-2x with 20 ml of PBS
4. Add 2 ml of Trypsin. Trypsinize for 3 min at 37C. Whack hard – you should see the cells coming down. (Important: never over-trypsinize the cells, so work quickly)
5. Add 10 ml of media and pipette up and down many times.
   (The serum in the media will neutralize the trypsin)
6. Observe cells to ensure they are not in clumps.
7. Add 22 ml of media to each new flask (we split 1 flask into 3 flasks)
8. Add 3 ml of cell culture-containing media to each flask, and pipette up and down to mix. Make sure the media covers the entire area of the flask. Put the cells into 37C with 5% CO2.

Estrogen treatment

I-1. Starve the cells

Grow the cells to 80% confluence. Observe cells under microscope to ensure that they are 80% confluent.

Aspirate off the growth media [DMEM/F12 media (Invitrogen/Gibco) supplemented with 5% FBS (Invitrogen/Gibco), penicillin (Invitrogen), streptomycin (Invitrogen), and gentamycin (Invitrogen)]

Wash cells 2x in each flask with 10 ml of 1x PBS (1^{st} Base).

Change into 20 ml of hormone-free media [clear, phenol-red free DMEM/F12 media (Invitrogen/Gibco) supplemented with 5% charcoal-dextran stripped FBS (Hyclone), penicillin, streptomycin, gentamycin, and L-glutamine (Invitrogen)] to hormone deplete the cells in a 150 mm diameter plate.

Grow cells in hormone-free media for a minimum of 72 hours.

I-2. Drug treatment

Add estrogen (17 beta-estradiol, E2, Sigma) into each plate to a final concentration of 100 nM, and incubate for 45 min.

Treat control cells with an equal volume and concentration of vehicle, ethanol (Merck), for 45 min.