Technical Data Sheet

Codex Estrogen Receptor Alpha Cell Line

Materials not included

BD Falcon™ 96-well Opaque White Microtest™ Plate	BD 353296
Dulbecco's Phosphate Buffered Saline (DPBS)	Sigma D8537

Cell culture materials not included

DMEM, high glucose, with glutamine	Invitrogen 11995-040
Fetal bovine serum	Invitrogen 26140-079
FETAL BOVINE SERUM, CHARCOAL STRIPPED	Invitrogen 217-500
Trypsin-EDTA solution (10x)	Sigma T4174
Puromycin	Clontech 8052-2
Phenol Red Free-DMEM	Invitrogen 21063-029

CELL CULTURE PROTOCOL.

THAWING AND PLATING CELLS (REQUIRES 1-3 DAYS)

1. Prepare complete cell culture medium consisting of 90% Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS) and 1μg/ml puromycin. Warm the medium to ~37°C.

2. Remove a vial of cells from the liquid Nitrogen tank. Wear safety glasses and always point the cap away from your face when opening.

3. Place the vial of cells in a 37°C water bath until just thawed (less than 5 minutes). Immediately transfer cells to a 10 cm cell-culture plate or a T25 flask with 9 ml of the appropriate culture medium (pre-warmed to 37°C).

4. Place the cells in a cell-culture incubator at 37°C with 5% CO_2 for 4 hrs.

5. After 4 hours replace the culture medium with appropriate fresh culture medium (pre-warmed at 37°C).

6. Place the cells back in the incubator for 1-3 days. The cells will not require feeding before they reach 80-90% confluence and are ready for expansion. Split the cells when they reach 80-90% confluence.

Note. It is very important that the cells DO NOT reach >90% confluence. Over-confluent growth can result in a significantly reduced response to ligands, and it may take several passages for the cells to recover to optimal stage.

SPLITTING AND AMPLIFYING CELLS (REQUIRES 1-3 DAYS)

1. Remove the culture medium and replace it with a volume of Dulbecco's Phosphate Buffers Saline without calcium and magnesium (DPBS) to adequately cover and wash the cells. Remove DPBS.

2. Add a sufficient volume of 1x trypsin-EDTA to just cover the cells (i.e. 1 ml for a 10 cm dish, 2 ml for a T25 flask, and 5 ml for a T75 flask) Rock the plate to make sure the cells are equally covered with the solution. Trypsinize the cells at room temperature for ~ 5 min. After 5 min, check the cells to ensure that they are coming off the dish/flask. Tap the dish/flask gently to aid in the process. Add enough serum-containing medium to give a volume of ~ 10 ml, and pipette the medium up and down through a 10 ml serological pipette ~ 4 times to obtain a single cell suspension. Remove a portion of the sample for a cell count.

Note. Trypsin is required to dissociate the cells during the process of passage. Cells may not be able to recover to an optimal stage if trypsin-free dissociation buffer is used.

3. For primary expansion from a frozen vial, reseed the total cell volume in a T150 flask. For routine cell passage, split the cells using a ratio of 1:4 – 1:10.

4. Observe the cells daily and harvest the cells when they reach 80-90% confluence (1-3 days). Cells do not need to be fed during this time. Do not allow the cells to grow over 90% confluence.

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FREEZING AND STORING THE CELLS

1. Remove cells from T75 flask by trypsinization as described above. Add 10 ml culture medium, and break the cell clumps via pipetting. Count cells using a hemocytometer.

2. Place cell suspension in a sterile centrifuge tube, and pellet the cells at ~ 200X g at 4°C for 5 min. Remove the medium, and resuspend the cell pellet in an appropriate volume of freezing medium (90% FBS and 10% DMSO) to give a cell density of 2.5 X 10^6 cells/ml.

3. Dispense the cells in 1 ml aliquots into cryo storage vials to give 2.5 X 10^6 cells/vial.

4. Freeze the cells in a cryo freezing-container overnight at -80°C.

5. Next day, transfer the cell vials to a liquid nitrogen tank for long-term storage.

ASSAY PROTOCOL

Note. Please finish reading the whole protocol before you start the experiment.

CELL PREPARATION

1. Culture the cells in growth medium containing 90% Phenol Red Free-DMEM, 10% CHARCOAL STRIPPED fetal bovine serum (FBS) and 1μg/ml puromycin for at least one passage before harvesting the cells.

2. Harvest cells when they reach 80-90% confluence in flasks. Trypsinize cells as described in Cell Culture Protocol above. Use 90% Phenol Red Free-DMEM, 10% CHARCOAL STRIPPED fetal bovine serum (FBS) and 1 μg/ml puromycin. Count a portion of the cells with a hemocytometer.

Note.1) It is very important that the cells DO NOT reach >90% confluence. Over-confluent growth can result in a significantly reduced response to ligands, and it may take several passages for the cells to recover to optimal stage. 2) Must use Phenol Red Free-DMEM and CHARCOAL STRIPPED fetal bovine serum at these two steps. Use regular DMEM and FBS will result in higher background and smaller assay window

3. The cell number needs to be optimized for each assay. Optimal assay conditions require a confluent monolayer of cells prior to the assay. Poly-D-Lysine coated plates are recommended to improve cell attachment. Plate 50K cells/well for a 96 well plate and 12K cells/well for a 384 well plate the day before the experiment. Add 100 μl/well of cell suspension to 96-well plates or 20 μl/well to 384-well plates.

4. Allow cells to attach by leaving the cell plates at room temperature for 30 minutes. Transfer the plates to a cell culture incubator and grow the cells overnight.

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