Protein Production UK

Expi 293 Protocols/ PEI MAX 40kDa

NB: Expi 293 Cells grow very well in Expi293™ Expression Medium

Valproic acid Sigma P4543
Sodium propionate Sigma P1880
Glucose Sigma G8769-100
PEI 40k Max Polyscience 24765-1

Thawing of cells:

Standard methods of thawing cells apply to Expi 293 cells. However; I wake cells by using a Pipette Gun set on gravity and slowly drawing and releasing cells in to the cryovial before transferring it into a 125ml Erlenmeyer (30ml maximum volume). After thawing the cells, incubate cells in a 37° incubator with a humidified atmosphere of 8% CO_2 in air on an orbital shaker at 125rpm.

Cells thawed in this manner, can also be introduced to attached format and once the flask is fully confluent, cells may be shaken gently and introduced to125ml Erlenmeyer flasks.

Freezing of cells:

Spin 30ml Expi 293 suspension cells in sterile Falcon tubes at 1200rpm for 10 minutes. Decant media and resuspend cells in 10ml of commercial cryo freezing media. Place 2ml of cells per cryovial and store at -80^{o}.

Cell Growth:

Expi 293 cells can reach up to 8 X 10^6/ml with 100% viability at times. in which case split the cells down to 3X10^5/ml on housekeeping days.

Otherwise cells are seeded between 4-5 X 10^5/ml on Monday's and Thursdays.

Expi 293 cells can be seeded as 30, 50, 100 (50ml cultures grown as 2X250ml Erlenmey flasks) & 300 ml cultures without any problems.

Transfection

Enhancers and PEI40K are stored frozen.

PEI, suspend 100mg of PEI Max 40K in 90ml of water. Stir using a PTFE-coated stirring bar. It should take less 5min. pH using sodium hydroxide or HCl to 7.00. Add water to 100ml. Filter 0.2um under the hood, aliquot, test and if good, freeze.

500mg Valproic acid (Sigma P4543), 10ml of cell media or HBSS, filter on 0.2um, freeze

1g Na propionate (Sigma P1880), 10 ml of cell media or HBSS, filter on 0.2um, freeze

4.5g glucose, 10 ml of cell media or HBSS, filter on 0.2um, freeze (or can buy from Sigma G8769)

Transfection protocol: ***30ml transfections***

Day 1: (Housekeeping days) determine cell count and viability. For transfection the following day, seed the cells between ≥1.0-1.5X10^6ml at ±95% viability.

Day 2: Determine cell count and viability, if cells are between 1.5-2X10^6/ml and at least 95% viable, transfections maybe performed. Return cells to incubator.

Transfection cocktail for 30ml scale up:

1) In a 15ml Falcon tube mix and 3ml OPTI-MEM media with 30ug plasmid DNA and 160ul of PEI Max 40kDa transfection reagent
2) Mix well
2) Incubate for 10 minutes at RT before adding to suspension cells.

To address glycosylation, use Kifunensine (after addition of transfection cocktail to cells) from a stock concentration of 1mg/ml. (30ul per 30ml scale up is 30ug inhibitor in total) and return cells to incubator.

Day 3: Addition of Enhancers

After 16-18 hours add **500u**l of Valproic acid **200ul** of Na propionate & **550ul** of glucose and return to incubator.

Day 5: Determine cell count and viability. Harvest media and spin in 0.5L Centrifuge bottle for 45minutes at 6000g. Filter sterilise using 0.5L 0.45um bottle top filter. Proceed to purification using OPPF-UK standard mammalian purification protocol for purification.

Transfection protocol: ***100ml scale up:***

Day 1: Determine cell count and viability and seed cells as 2X 50ml cultures in250ml Erlenmeyer flasks at seeding density of ≥1.0-1.5X10^6ml at ±95% viability.

Day 2: Determine cell count and viability, if cell count is between ≥ 1.5-2X10^6/ml and at least ±95% viable, transfections maybe performed. Return cells to incubator.

Transfection cocktail for 100ml scale up**:**

1) In a 15ml Falcon tube mix 10ml OPTI-MEM media with 100ug plasmid DNA and 540ul of PEI Max 40kDa transfection reagent
2) Mix well
3) Incubate for 10 minutes at RT before adding to suspension cells.

To address glycosylation, use Kifunensine (after addition of transfection cocktail to cells) from a stock concentration of 1mg/ml. (100ul per 100ml scale up is 30ug inhibitor in total) and return cells to incubator.

Day 3: Addition of Enhancers

After 16-18 hours add **1700u**l of Valproic acid **650ul** of Na propionate & **2000ul** of glucose and return to incubator.

Day 5: Determine cell count and viability. Harvest media and spin in 0.5L Centrifuge bottle for 45minutes at 6000g. Filter sterilise using 0.5L 0.45um bottle top filter. Proceed to purification using OPPF-UK standard mammalian purification protocol for purification.

Transfection protocol: ***300 ml scale up:***

Day 1: Determine cell count and viability and seed cells in squatty roller bottle with 300ml culture volumes at a density of ≥1.0-1.5X10^6ml with ±95% viability.

The lid used are vented red caps. Do not use the orange lids with the bottle. Day 2: On day of transfection, determine cell count and viability, if cell count is between ≥1.5-2X10^6/ml and ±95% viable, transfections maybe performed. Return cells to incubator.

Transfection cocktail for 300ml scale up**:**

1) In a 50ml Falcon tube mix 30ml OPTI-MEM media with 300ug plasmid DNA and 1600ul of PEI Max 40kDa transfection reagent
2) Mix well
3) Incubate for 10 minutes at RT before adding to suspension cells.

To address glycosylation, use Kifunensine (after addition of transfection cocktail to cells) from a stock concentration of 1mg/ml. (300ul per 300ml scale up is 30ug inhibitor in total) and return cells to incubator.

Day 3: Addition of Enhancers

After 16-18 hours add **5m**l of Valproic acid **2ml** of Na propionate & **5500ul** of glucose and return to incubator.

Day 5: Determine cell count and viability. Harvest media and spin in 0.5L Centrifuge bottle for 45minutes at 6000g. Filter sterilise using 0.5L 0.45um bottle top filter. Proceed to purification using OPPF-UK standard mammalian purification.