Sample Calculations for:

**High yield expression of recombinant human proteins with the transient transfection of HEK293 cells in suspension**

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**1.2.6 Sample calculation:**

The density of HEK 293F cells in the donor culture is 2.24 × 106 live cells/ml.

The new culture will be 30 ml with a final density of 0.3×106 live cells/ml. To calculate the amount of donor culture, multiply to final density by the culture volume, and divide this figure by the density of the donor culture:

(0.3×106 live cells/ml \* 30 ml)/ 2.24×106 live cells/ml = 4.0 ml

Hence, for this particular cell passage, the amounts of materials needed are as follows:

4 ml of cells in stock suspension culture, 3 ml of Medium B and 23 ml of Medium A.

**3.1.1 Sample calculation:**

Cells density in the stock suspension culture = 2.24×106 live cells/ml with viability > 95%.

Cells density for the transfection volume of 50 ml = 3.0×106 live cells/ml.

Therefore, volume of suspension culture required =

(50 ml × 3.0×106 live cells/ml)/(2.24×106 live cells/ml) = 67 ml

**3.1.3 Sample for a 50 ml transfection volume:**

Withdraw, 5 ml of prewarmed Medium B (10% of final volume).

Withdraw, 45 ml of prewarmed Medium A and add to the 5 ml of Medium B to prepare fresh culture medium.

Withdraw 10% of the fresh culture medium (5 ml) medium in a separate sterile 15 ml falcon tube. This will be added back in Step 3.1.12.

**3.1.6 Sample Calculations:**

DNA solution 0.5 μg/μl:

DNA stock concentration = 4 μg/μl (in sterile 10 mM Tris, pH 8.0)

For a 50 ml transfection (100 ml final culture volume), amount of DNA required = 3 μg/ml × 50 ml = 150 μg. Add 10% for pipetting error = 165 μg.

Therefore, withdraw 165 μg/(4 μg/μl) = 41 μl of sterile DNA.

Dilute the stock DNA solution at 0.5 μg/μl

Determine the final volume: (41 μl × 4 μg/μl) / 0.5 μg/μl= 328 μl. Subtract the volume of the DNA (41 μL) = 287 μl. Withdraw 287 μl of fresh culture medium and add to the aliquot of 41 μl of stock DNA solution to prepare a working solution containing 0.5 μg/μl DNA.

PEI solution 0.5 μg/μl:

PEI stock concentration = 1 μg/μl. For a 50 ml transfection (100 ml final culture volume), the required quantity for a final 9 μg/ml transfection of PEI is = 450 μg of PEI at 0.5 μg/μl.

To account for pipetting error, we increase the volume by 10%. Dilute 450 μl + 10% pipetting error = 495 μl of PEI stock with 495 μl of fresh culture medium = 990 μl of PEI solution.

Now we can adjust the final culture volume to 50 mL. Remember that we withheld 5 ml, now we can calculate how much medium to add back to the culture before transfection. 5 ml – (0.3 mL 0.5 μg/μl DNA + 0.9 mL 0.5 μg/μl PEI )= 3.8 ml.

Thus, the culture media volume excluding the volume of the DNA and PEI solutions = 48.8 ml

**7.1.2 Sample calculation for a 50 ml transfection volume:**

Withdraw, 5 ml of prewarmed ExCell 293 medium (10% of final volume).

Withdraw 41 ml of prewarmed FreeStyle medium and add to the 5 ml of ExCell 293 Serum-Free Medium to prepare fresh culture medium.

Withdraw 10% of the fresh culture medium (4.6 ml) medium in a separate sterile 15 ml falcon tube.

Add 4 ml of the sterile amino acid mixture solution to the 41.4 mL of Freestyle and ExCell media