Transfection Protocol for COS 7 Cells

**Growth Media**

DMEM H-Glucose( 4.5g/L) Gibco brand (500mL)

10% FBS (FBS from Benchmark 100% stock) (add 50mL to DMEM)

1x Antibiotic-Antimycotic (AA from Gibco 100X stock) (add 5mL to DMEM)

**Plates**

12-well tissue culture treated plates (Costar 3512)

**Day 1** (Plate Cells)

 Plate 65,000 cells per well in a 12-well plate in 1mL of growth media (65,000 cells/mL)

 Place plate in a 37°C incubator (5% CO2) for 18-24 hrs

**Day 2** (Transfection Day)

Transfect 200-800ng of DNA in each well. For values less the 200ng use the desired amount of DNA and the rest pcDNA.

Transfections require a 4:1 ration of fugene(uL):DNA(ug). (i.e. 1ug of DNA: 4uL of Fugene)

1. Remove the fugene stock bottle from the fridge and place it on your bench to warm up to room temp.
2. Remove the maxipreped DNA stocks from the 4°C incubator and place it in an ice bucket.
3. Make a dilution of the maxiprepped DNA with **sterile ddH2O**, and place the diluted stock on your bench at room temp. The diluted stock should be 100 ng/μL.
4. Aliquot the appropriate volumes of diluted DNA into a 1.5mL epindorf tube for each transfection condition. DO NOT put the aliquoted amounts of DNA into the ice leave at room temperature.
5. Take the fugene and serum-free DMEM (SF DMEM) both at room temperature into the tissue culture hood.
6. Briefly vortex the fugene bottle.
7. Aliquot the amount of SF DMEM into a 15mL or 50mL conical tube.
8. Pipet the appropriate amount of fugene for the transfection into the center of the SF DMEM. DO NOT pipet it onto the side of tube. Pipet up and down 1 time.
9. Gently shake once or twice to mix and let sit for 5 minutes.
10. Retrieve 1.5mL epindorf tubes of aliquoted DNA and place in the tissue culture hood.
11. After 5 minutes has passed, add appropriate amount of fugene/SF DMEM mix into the each 1.5ml epindorf tube. As adding fugene/SF DMEM pipet up and down **once** to mix.
12. Let sit for 15-20 min. Stager the time between additions of fugene/SF DMEM to each sample so each sample sits for only 15-20 min prior to addition to cells.
13. After 15 min. add 50μL of transfection reaction with DNA into each well from the previous plating day. Shake plate lightly to mix.
14. Place plate back in 37°C incubator (5% CO2) for 24-48 hrs.

**Below is an example transfection with a 12 well tranfection sheet**

Example below uses 500ng per well in a 15 well transfection in two 12 well plates.

For each transfection enough is made for 4 wells as shown in the calculation sheet below. Enough sample is made for 4 wells, but only 3 wells will be used in the transfection to reserve room for pipetting error. In the example transfection below there are 5 separate conditions, each condition contains enough sample for 4 wells each, for a total of 20 wells. 2 extra wells are added for pipetting error to the Fugene/SF DMEM mixture for a final of 22 wells.

Each condition contained 200ng of desired DNA and the rest was supplemented with pcDNA for a final of 500ng total.

Tubes 1-5 were first aliquotted with the appropriate amount of desired DNA and pcDNA.

In the hood combine 946μL SF DMEM and 44μL Fugene. Shake and let sit for 5 minutes.

After 5 minutes add 180 μL of the SF DMEM/Fugene mix to each of the aliquoted DNA tubes

Let sit for 15-20 minutes.

Add 50uL to each of 3 wells to run the sample in triplicate. There will be excess sample in the tube that will be waste.

 