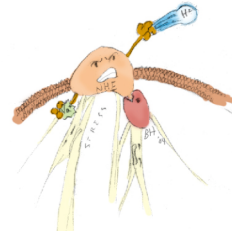


## Basic Transfection Protocol



**Introduction:** Transient transfection utilizes a mixture of liposomes and DNA to shuttle the plasmid DNA across the membrane. These protocols are specific to CCL39 cells but have worked with good success in a number of other cell lines. Always determine the ration, total mass of DNA and time for expression for your needs before assuming any one protocol will work for your needs. Ratios and mass will need to be screened for optimal expression in each case. We have found a 80% transfection efficiency using a GFP containing vector as our marker. About 70% of the transfected cells are strong expressers the rest are medium to weak.

*This protocol is based for transfecting the day before starving the cells in a serum free media overnight and the next day stimulating the cells for signaling experiments. Timing may vary for your needs.*

### Basic Plan

- 3:2 ratio of  $\mu\text{l}$  Fugene-6 to  $\mu\text{g}$  DNA
- 7.5  $\mu\text{g}$  Plasmid DNA

### Materials

- Optimem
- PBS
- Serum Free ("Base") or Low Serum Media (0.5% FBS)
- 1 mg/ml Plasmid DNA
- Sterilized Tips and Microfuge Tubes

### Timeline

- Day 1 - Morning Transfection
- Day 1 - Evening Media Change
- Day 2 - Morning Change to Serum Free ("Base") Media
- Day 2- Experiment

### Procedure

#### 1. Preparation of Fugene-6 - DNA complex (Use sterile tips and microfuge tubes)

- Conduct the entire procedure in the sterile hood.
- Prepare Sterile Microfuge tubes (label tubes)
- Add the indicated amount of Optimem to each tube
- Carefully add the fugene-6 to each tube (READ THE FOLLOWING)
  - Use a new tip each time
  - Limit the time the fugene-6 stock tube is open to reduce evaporation of the solvent
  - Add the transfection reagent directly to the optimem: Put the tip directly into the optimem do not allow the reagent to touch any other plastic other than the pipette tip. Fugene-6 will bind to the plastic and limit the effectiveness of your transfection.
- Tap, do NOT vortex each tube and incubate for 10 min at room temp
- Directly add the DNA to the mixture AFTER the incubation
- Mix by tapping the tube- do not vortex
- Incubate for 20 - 30 min. at room temperature.

## Basic Transfection Protocol

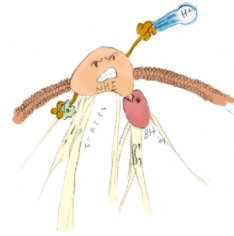


Table 1: Preparation of DNA/Fugene-6 Suspension. Varying DNA concentrations at a constant 3:2 ratio.

	Optimem ( $\mu$ l)	1 mg/ml Stock DNA ( $\mu$ l)	Final DNA Mass	Fugene-6 ( $\mu$ l)
35 mm dish or one 6 well plate	81.2	7.5	7.5 $\mu$ g	11.3
60 mm plate	139.2	12.05	12.05 $\mu$ g	19.2

\* If doing identical repeats, multiply by the volumes needed instead of preparing repeat tubes, this reduces the surface area of plastic to interfere with transfection

### 2. Transfection of Cells

- Transfecting cells when 40-70% confluent works best. Depending on the rate of growth, cells will be 80-90% confluent on day 2. *Do NOT transfect confluent cells, they do not take up the DNA well and do not express protein to reasonable levels.*
- While incubating the DNA/Fugene-6/Optimem mixture, remove media from cells.
- Rinse cells twice with about 2 ml of Ca<sup>2+</sup>/Mg<sup>2+</sup> free (CMF) - PBS
- Add Optimem to rinsed cells
  - 1.4 ml for each well of a 6 well dish or each 35 mm culture dish
  - 2.4 ml for each 60 mm dish
- Add the DNA/Optimem/Fugene-6 mixture directly (slowly and dropwise) onto cells while swirling media in cells to mix. **DON'T** drop in the mixture and then mix.
- Incubate overnight
- The next morning, remove media, rinse with CMF-PBS, and add 1.5/2.5 ml low serum (0.5% FBS) media to 6well plates-35 mm dishes/60 mm dishes.