Protocol for protein expression in 293F cells by transient transfection

Materials.

- Cells: HEK 293 Freestyle
- Cell culture media: 293 Freestyle expression media (Invitrogen)
- Transfection media: Opti-MEM (Invitrogen)
- Transfection reagent: 293Fectin (Invitrogen)
- Flasks: 2-liter cell culture flask with baffled bottom (Nalgene)
- Filters: 0.22 µm 50 ml Steriflip (Millipore, cat# SE1M179M6); 0.22 µm 500 ml Stericup (Millipore, cat# SCGPU05RE)
- Incubator: Shaker incubator, 120 rpm, 37°C, 8% CO2

Procedure.

1. Passage cells in 293 Freestyle expression media through continuous log-growth phase twice before use. Final viable cell density should be in the range of about 1x10e6 cells/ml and cell viability ≥96% for transfection.
2. Prepare DNA/Fectin mixture as follows:
    a. Dilute 1ml of 293Fectin into 25ml of Opti-MEM by gently mixing and incubating for 5 min at room temperature (RT).
    b. Dilute 500µg of the total DNA (250µg each of heavy and light chain) into 25ml of Opti-MEM, and sterile filter (0.22 µm Steriflip).
    c. Add Fectin/Opti-MEM solution dropwise into DNA/Opti-MEM solution while mixing gently.
    d. Incubate the mixture for 20 minutes at RT.
3. Add DNA/Fectin mixture (50 ml) dropwise to 1L of 293 Freestyle cell suspension containing ~1x10e6 cell/ml) while mixing gently.
4. Incubate transfection culture in a 37°C, 8% CO2 shaker incubator for 5 days.
5. Harvest expressed IgG by centrifuging the suspension (3000*g* for 25 minutes) and filtering the supernatant through a 0.22 µm filter.