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## Transient Transfection Protocol

**Step 1, Preparation of cells:**

1. Approximately 18-24 hours before transfection, plate ~$5x10^4$ adherent cells or ~$5x10^5$ suspension cells per well to obtain 50-70% confluence on the following day.

**Step 2: Preparation of the Turbofectin 8.0/DNA Complexes (prepare immediately prior to transfection):**

1. Dilute 0.5 µg of DNA in 50 µL of Opti-MEM I (Gibco 51985). Vortex gently.
2. Add 1.5 µL of Turbofectin 8.0 to the diluted DNA (not the reverse order) and pipette gently to mix completely.
3. Incubate for 15 minutes at room temperature.

Note: We recommend starting with the ratios of Turbofectin 8.0 and DNA listed in Table 1; however, subsequent optimization may further increase the transfection efficiency.

**Step 3, Transfection:**

1. Gently add the Turbofectin 8.0 / DNA mixture from step 2 drop-wise to each well (already containing about 500 µL of culture medium). Gently rock the plate back-and-forth and from side-to-side to achieve even distribution of the complexes. Incubate at 37°C for 24-48 hrs.

Table 1. Recommended starting transfection conditions for Turbofectin 8.0

| Tissue Culture Vessel | Growth area, $cm^2$/well | µg of DNA | Ratio of Turbofectin to DNA |
|---|---|---|---|
| 96-well plate | 0.35 | 0.1-0.3 | 3:1 |
| 24-well plate | 2 | 0.25-1.25 | 3:1 |
| 12-well plate | 4 | 0.5-2.5 | 3:1 |
| 6-well plate | 9.5 | 1-5 | 3:1 |
| 35 mm plate | 8 | 1-5 | 3:1 |
| 60 mm plate | 20 | 2-10 | 3:1 |
| 100 mm plate | 60 | 5-15 | 3:1 |

## Stable Transfection Protocol

1. Perform the transfection as described above (protocol for transient transfection).

2. 24 hours post-transfection, passage the cells (at 1:10 or higher dilution) into fresh growth medium containing selective agent (the correct dose needs to be determined by a killing curve experiment).

Note: A mock transfection should be performed in parallel as a control.

3. Grow and passage the cells as necessary, maintaining selection pressure by keeping the selective agent in the growth medium. After 1-2 weeks, a large number of the cells will be killed; the cells that remain growing in the selective medium have retained the expression plasmid, which stably integrates into the genome of the targeted cells. Monitor the mock control to ensure the cells are dying.