TRANSFECTION OF SUSPENSION CELLS

The following modification of the DDMC transfection procedure has been used successfully for transfecting lymphoid cell lines in suspension. It is derived from the protocol of DEAE-dextran by Fujita et al. (1986).

Additional Materials Suspension TBS (STBS) solution, 37°C Lymphoid cell line or eukaryotic cells of choice 10 mg/ml DDMC in STBS solution Complete medium without serum (appropriate for the cell line) Sterile 50-ml polypropylene tube Beckman JS-4.2 rotor or equivalent

If necessary ----------

1. Ethanol precipitate the DNA to be transfected in a microcentrifuge tube.
Air dry the pellet in a tissue culture hood by inverting the tube on Kimwipes.
Resuspend the DNA in 0.5 ml STBS solution. Approximately 10 µg of DNA should be transfected into 2×10^7 cells. As with several other parameters of DDMC transfection, this should be optimized for the particular cell line.

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2. Collect 2×10^7 cells in a sterile 50-ml polypropylene tube by centrifuging 5 min at 640 × g (1500 rpm in a JS-4.2 rotor), room temperature, and discard supernatant.
Cells should be growing logarithmically on the day of transfection.

3. Wash the cells by resuspending in 5 ml of 37°C STBS solution and collecting as in step 2.

4. Prepare a solution from DDMC (as 10 mg/ml of DEAE-dextran) containing twice the intended final concentration of DDMC in STBS solution. Add 0.5 ml of this solution to the 0.5 ml DNA solution of step 1 and mix. Resuspend the cells in 1.0 ml DNA/DDMC solution. The optimal DDMC concentration to use will vary with cell line between approximately 100 and 500 µg/ml(as 10 mg/ml of DEAE-dextran). It is convenient to have a 10 mg/ml stock of DDMC in STBS solution that is diluted for use in the transfection. Supplement 3 Current Protocols in Immunology 10.14.3 Transfection Using DDMC.

5. Incubate the cells for 30 to 90 min. Tap the cells every 30 min to keep them from clumping.
Precise time for incubation depends on the cell line and should be optimized.

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6. Add DMSO to the cells dropwise to 10% final. Mix the contents of the tube while adding the DMSO to ensure that the DMSO is rapidly mixed with the cells.

7. Incubate 2 to 3 min at room temperature. Add 15 ml STBS solution to the cells.
Addition of the STBS solution dilutes the DMSO so that the DMSO “shock” does not continue past 2 to 3 min.

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8. Collect the cells by centrifuging 5 min at 300 × g (1000 rpm in a JS-4.2 rotor), and discard supernatant.

9. Wash the cells by resuspending in 10 ml STBS solution and collecting as in step 8, then wash with 10 ml complete medium without serum.

10. Resuspend the cells in complete medium to the desired concentration (generally between 2-10 × 10^5 cells per ml) and incubate until ready to harvest and analyze.
The cells should be resuspended to a concentration that will allow them to grow until they are harvested.Generally maximal expression of transfected DNA is ~48 hr after the transfection.

Suspension TBS (STBS) solution
25 mM Tris⊕Cl, pH 7.4
137 mM NaCl
5 mM KCl
0.6 mM Na2HPO4
0.7 mM CaCl2
0.5 mM MgCl2
Make up in distilled H2O, filter sterilize, and store at 4°C. A 10· stock of this solution can be made that is diluted to 1· with sterile distilled H2O prior to use.

Tris-buffered saline (TBS)
For 100 ml, add 10 ml solution A to 89 ml H2O. While stirring rapidly, add 1 ml solution B slowly, drop by drop. Filter sterilize and store at 4°C.

Solution A:
80 g/liter NaCl
3.8 g/liter KCl
2 g/liter Na2HPO4
30 g/liter Tris base
Adjust pH to 7.5
Filter sterilize
Store at ↕20°C

Solution B:
15 g/liter CaCl2
10 g/liter MgCl2
Filter sterilize
Store at ↕20°C

Make certain that the stock TBS solution is mixed well just before use by bringing the solution into and out of a sterile 10-ml pipet several times.

Table 1. Recommended number of cells per culture vessel for transfection

Culture format	Adherent cells Number to seed the day prior to transfection*†	Suspension cells Number to seed on day of transfection*	Volume of medium (µl)
96-well plate	0.5–2.0 x 10^4	0.5–2.0 x 10^5	100
48-well plate	1.0–4.0 x 10^4	1.0–3.5 x 10^5	150
24-well plate	2.0–8.0 x 10^4	2.0–7.0 x 10^5	350
12-well plate	0.4–2.0 x 10^5	0.5–1.5 x 10^6	800
6-well plate	0.9–4.0 x 10^5	1.0–3.5 x 10^6	1600
60 mm dish	2.0–8.0 x 10^5	2.5–7.5 x 10^6	4000
100 mm dish	0.5–2.5 x 10^6	0.5–2.0 x 10^7	7000

** Actual values depend on cell type and size.*

† The volume of medium used to seed adherent cells the day before transfection is not critical. When seeding adherent cells, use a volume of medium suitable for your cell culture format.