Transient Transfections with Lipofectamine-Plus

1 Seed 400 000 cells per well of a 6-well plate (40 000 cells/ cm^2)

2 Next day: (cells should be 80% confluent): Prepare transfection mix:
Per well (per 1 ml of transfection mix = for 10 chambers of an 8-chamber LabTek):

2.1 Dilute the DNA in 100 µl serumfree medium, add Plus-reagent and mix (do not vortex). Incubate for 15 min at room temperature.
(The total amount of DNA should be the same for all samples; if necessary add an unrelated control plasmid. If you want to control the transfection efficiency, you can add a constant amount of a GFP-vector, e.g. EGFP-C1 from Clontech).

2.2 Dilute the Lipofectamine in 100 µl serumfree medium.

2.3 After the incubation of the DNA solution with the Plus-reagent, add the Lipofectamine/medium solution (100 µl), mix carefully and incubate for 15 min at room temperature.

2.4 Add 800 µl serumfree medium (gives a total of 1 ml) and add the transfection mix to cells, which have been washed with serumfree medium shortly before.

2.5 Incubate the cells for the appropriate time at 37°C in the incubator (incubation time depends on the cell type).

2.6 After the incubation, remove the transfection mix and add the normal serum-containing medium.

3 Analyze or extract the cells one or two days after the transfection. You can control the transfection efficiency of GFP-transfected cells by fluorescence microscopy or flow cytometry.

Conditions (for one well of a 6-well plate = for 10 cm^2)

Cell type	Medium	DNA	Plus-R.	Lipofectamine	Incubation	efficiency
HeLa	DMEM	1.5 µg	5 µl	3 µl	7 h	50 - 70 %
293	DMEM	1 µg	4 µl	3 µl	3 - 5 h	70 - 100 %
CHO	MEM-alpha	1 µg	5 µl	2 µl	3.5 h	50 - 70 %
HUVEC	M 199	1.5 µg	8 µl	4 µl	2 - 3 h	10 - 20 %