Transfection of mammalian cells using Ca-phosphate, standard procedure (from Halle)

-for further information see: Maniatis and original paper as referenced there.

DNA: Column purified. (EtOH/NaAC precipitation before transfection; bring DNA under hood in 70 % EtOH, dry the pellet under the hood and dissolve it in sterile cell culture grade water). For cotransfection of resistance plasmid, use 10 x lower **molar** amount of resistance plasmid compared to plasmid of choice. Cotransfection of several plasmids/plasmid mixes is possible. The concentration of selective agent has to be established for every cell type.

Cell density: Depends on growth rate of cells. Ideally, cells should be plated 24-48 h before transfection. At the time of transfection, they should be 30-50 % confluent. Fast growing cells (doubling time 12 h or less) should be transfected at even lower density.

Reagents:

-2x DNA-precipitation buffer:

50 mM Hepes pH 7,05

1,5 mM Na_2HPO_4

10 mM KCl

280 mM NaCl

12 mM Glucose -> filter sterile, check pH carefully !!

-2M **$CaCl_2$** (sterile)

-Buffer for glycerol shock **:**

1,35 ml Glycerol

3,15 ml Cell culture grade water

4,5 ml 2x DNA-precipitation buffer. -> filter sterile, prepare fresh each time !!

For preparation of all solutions use cell culture grade reagents only!

Procedure:

1. Plate the cells 1-2 days before transfection in a 10 cm dish (with 15 ml medium). If you want to analyse transfection by immunofluorescence, place sterile coverlips into dish before adding cells.
2. 2 hours before starting, change the medium
3. Composition of 1,5 ml calcium phosphate /DNA precipitate solution (for one 10 cm dish) :
   750 µl 2x DNA precipitation buffer
   60 µg DNA
   93 µl 2M $CaCl_2$
   add 1.500 µl cell culture water
4. Add the calculated amount of water to a 2ml Eppi and mix it carefully with the DNA.
5. Add 93 µl 2M $CaCl_2$ (Do not mix!)
6. Slowly add 750µl 2x DNA precipitation buffer (dropwise!) to the tube. Mix very gently by bubbling or mild swirling
7. Incubate the mixture for **20 min**. under the hood
8. Mix the mixture very, very! gently and add it slowly and dropwise to the cells. During this procedure move the dish carefully to mix the medium with the precipitate (after addition check the cells under the microscope : a fine white precipitate should be visible)
   -> incubate for **6 hours** in the incubator at 37°C
9. Wash the cells 3-4 times with 6 ml medium without FCS
10. Glycerol shock procedure: add 3,5ml Glycerol shock buffer to the dish and incubate at room temperature (for example **2,5 min** for MCF7-cells, microscope check!) (length of incubation depends upon the degree of tolerance of the cell line and should be tested). Immediately wash the cells 3x with medium without FCS.
11. Add 15 ml medium (with FCS) containing 1xP/S and incubate at 37 °C

For stable transfections, add selective agent early next morning. Keep in mind, however, that cells must not be confluent when selection starts. In case cells are confluent, trypsinize and replate 30-50 % of cells per dish.

For transient transfections: expression of protein may start as early as 7 h after transfection and persists for up to 120h. Typical time points for analysis are however between 24-72 h. Analyze expression after several time points by immunofluorescence or western blotting or PCR, whatever is appropriate.

Remarks:

Starting with a new cell line /experiment, consider the following parameters for optimization: a) Amount of DNA (40-60 µg /10 cm dish), B) cell density (10-80 %), C) duration of glycerol shock (0-10 min.).

Some cells may not tolerate glycerol shock. Test procedure without.

For stable transfections, carry out the following controls :

1. 1 dish without CaPO4 /DNA precipitate
2. 1 transfected dish without selection medium
3. 1 untransfected dish with selection medium

Transfektionsansatz

	H_2O µl	**DNA µg**	**2M $CaCl_2$ µl**	**2xPP µl**	**Gesamt µl**	**Fläche (cm2)**
24-well	28	2,5	4	32	64	1,86
12-well	56	5	8	64	128	3,6
6-well	140	12,4	20	160	320	8,96
60-mm	280	24,8	40	310	630	22,10
100-mm	**840**	**77**	**120**	**960**	**1920**	60,10

- **Bei Kotransfektionen – Plasmidanteile 50%/50% verwenden (z.B. 100 mm Platte - 38,5 µg + 38,5µg)**

Mediumwechsel vor Transfektion (1-2h)

	Fläche (cm^2)	**Medium (ml)**
24-well	1,86	0,250
12-well	3,6	0,500
6-well	8,96	3,0
60-mm	22,10	5,0
100-mm	60,10	10