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Calcium Phosphate Transfection

This protocol results typically in >80% of 293 cells expressing transfected protein. Transfection efficiency is reliably better than lipofectamine but sometimes inconsistent so use GFP control and check visually for precipitate before changing medium post-transfection. Calcium phosphate transfection can be toxic for some cell types (HOS and to some extent HeLa). Virus produced by calcium phosphate transfection in 293 cells is around two-fold higher titer than virus produced by lipofection.

- 2.5 M $CaCl_2$
- 2X HBS Buffer (pH 7.05-7.09)
- Sterile ddH_2O
- 5 mL polystyrene tubes (BD Falcon cat#352054)

2.5 M $CaCl_2$, 500 mL

$CaCl_2$•2 H_2O (CAS 10035-04-8)	183.7 g
Deionized H_2O	Add to 500 mL

Sterile filter. Store in tissue culture 4˚C next to 2X HBS.

2X HBS Buffer, 1 L

NaCl (CAS 7647-14-5)	16.4 g
HEPES (CAS 7365-45-9)	11.9 g
Na_2HPO_4 (CAS 7558-79-4)	0.21 g
ddH_2O	Add to 1,000 mL

Divide 1,000 mL into 3-4 volumes. pH fractions to a range of 7.05 – 7.09 with NaOH. Sterile filter, keep at 4˚C.

Test pH'ed HBS stocks for formation of precipitation and transfection efficiency with a GFP expressing plasmid. pH range of pH 7.05 – 7.09 is preferred. Changes in size of precipitate are observed between HBS stocks of different pH values.

	T25	**12 well**	**6 well**	**10 cm plate**
DNA	12 μg	2 μg	4 μg	36 μg
H_2O up to	225 μL	35 μL	70 μL	675 μL
$CaCl_2$	25 μL	5 μL	10 μL	75 μL
2X HBS	250 μL	40 μL	80 μL	750 μL

PROTOCOL

1. In tissue culture hood, dilute DNA in sterile, ddH_2O to the final volume appropriate for the size of the dish. Use 5 mL polystyrene tubes.

2. Add appropriate volume of $CaCl_2$.

3. Add appropriate volume of 2X HBS slowly and drop-wise while "bubbling" $CaCl_2$/water/DNA solution with 2 mL pipet.
   To bubble, press expeller button on pipet aid with tip submerged in liquid. Bubble throughout addition of HBS and for ~30 seconds after last drop is added.

4. Let solution incubate 5 min. rm. temp.

5. Add dropwise to cells. Swirl gently to mix.

6. Change medium 6-7 hrs post-transfection. Before changing medium, check visually for precipitate with microscope on high magnification. Precipitate should look like small grains of sand, usually most evident in spaces between cells.

NOTES

- HBS can go off after about 6 months.
- During transfection, keep water, $CaCl_2$ and 2X HBS cold.
- Don't draw directly from stock bottles of $CaCl_2$ and 2X HBS to prevent contamination. Take aliquots!
- Master mixes for $CaPO_4$ transfection do not work as effectively. Make one transfection reaction per well or dish, even if DNA is identical.
- Always add in GFP control to check transfection efficiency. Transfect appropriate amount of GFP expressing plasmid and check signal under fluorescent microscope 24-48 hrs post-transfection.
- Cells should be 80-90% confluent and evenly distributed for transfection. Important for virus production.
- For large transfections, break into groups of 3-4 to minimize warming of solutions and extended incubation of reactions.