Calcium Phosphate Transfection of adherent cells

Reagents

2X HBS

280mM NaCl	8.18g/500ml	pH with NaOH
10mM KCl	0.37g/500ml	After adjusting volume with ddH_2O, filter
1.5mM Na_2HPO_4	0.106g(anhydrous)/500ml	sterilize with 0.22μm filter
12mM dextrose (glucose)	1.08g/500ml	
50mM HEPES pH7.05	5.96g/500ml	

2M $CaCl_2$

Make fresh weekly
2.94g $CaCl_2$ / 10ml H_2O
Filter sterilize

Sterile deionized or distilled H_20

Medium with FBS (DMEM, **NOT RPMI 1640!!!!**)

Plasmid DNA

Vortex

15cc tubes

Protocol

Note: The following protocol is written for transfection of cells in a 10cm dish. Volumes can be increased or decreased accordingly with need.

1. Split cells, placing 9ml of cells + medium in a 10cm dish. Depending on the time of assay, plate sparsely or more densely. (For HeLa cells, I split 1:8-1:10 for assay at 40hrs post-transfection, 1:20 for assay at 72 hrs post-transfection; for stables, split more sparsely, e.g. 1:40).

2. Prepare DNA/$CaPO_4$ mix for transfection 4-6hrs after plating.

Note: most protocols call for plating the cells the day before. I have found that plating cells 4-6hrs prior to transfection improves the efficiency of transfection, in some cases greatly. This is likely due to the cells being not completely spread out on the plate, thereby providing more surface area for contact with the precipitate and perhaps a higher degree of phagocytosis by extension of lamellopodia and pseudopodia.

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3. Combine in a sterile tube (1.5ml screw-cap eppendorf is good):

- 10-20μg of total DNA. You can vary the level of expression of the gene of interest by varying the amount of the plasmid of interest at this step. For instance, for non-saturating levels of expression of internalized proteins, I find 0.5μg of pCDM8 plasmid per 10cm dish of HeLa cells is appropriate; 10μg results in massive overexpression. Make up the difference to at least 10μg by adding empty expression vector or other carrier DNA.
- 62μl of 2M $CaCl_2$
- H2O to 500μl

Mix well.

4. Add this mixture to 500μl of 2XHBS at room temperature in a 15cc tube. Vortex well.

Note: For transfection of multiple plates, you can combine samples at steps 3 and 4 - i.e., you don't need to have 1 tube/plate specifically if you are transfecting multiple plates with the same thing.

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5. Let DNA/$CaCl_2$/HBS mixture sit at room temperature for 10-30 minutes.

6. Mix well again, and then sprinkle the entire mixture over the plate of cells. Let the cells sit overnight in the incubator.

7. The next morning, swirl the plates gently and remove the medium. Add 10ml fresh warm medium to the plates and grow in the incubator until ready for assay.

The following volume adjustments can be made:

For 6 well plates: use 3-5μg DNA total; cells in 2.25ml medium; 125μl total volume of DNA/$CaCl_2$ mix (with 16μl 2M $CaCl_2$); 125μl 2XHBS

For 12 well plates: use 1.5-2.5μg DNA total; cells in 1.12ml medium; 62.5μl total volume of DNA/$CaCl_2$ mix (with 8μl 2M $CaCl_2$); 62.5μl 2XHBS

For 15cm plates: use 25-40μg DNA total; cells in 22.5ml medium; 1.125ml total volume of DNA/$CaCl_2$ mix (with 155μl 2M $CaCl_2$); 1.125ml 2XHBS