TED MAZZONE'S PROTOCOL FOR STABLE TRANSFECTION OF J774 CELLS BY CALCIUM PHOSPHATE CO-PRECIPITATION

Dr. Theodore Mazzone
Professor of Medicine, University of Illinois at Chicago
Chief, Section of Diabetes and Metabolism MC 797
1819 W. Polk St, Chicago, IL 60612-7333
(312) 996-7989 phone; (312) 413-0437 fax
tmazzone@uic.edu

1. Start with cells at 60% confluency in 100 cm dishes. The cells should not be too confluent when you add the selection agent as selection will be inefficient.

2. Preparation of the Ca_2PO_4-DNA solution. Each dish requires 25 µg DNA in 1 ml Ca_2PO_4-DNA solution. [For co-transfection use 25 µg DNA and 25 ng plasmid containing the selectable gene marker.]

   Prepare solution A and B separately in 15 ml sterile tubes.

Solution A		Solution B	
DNA/H_2O	100 µl	2M $CaCl_2$	63µl
2xHeBS	500 µl	H_20	337 µl

   Dropwise add solution B to A while gently bubbling air through solution A.
   (Use a 1 ml pipette with cotton filter to bubble air.)

   Cap tube tightly. Let stand at room temperature for 30 min. A fine precipitate should begin to form after 5-10 min. It is very important that the precipitate that forms is very fine--just barely visible to the naked eye. This varies somewhat—it is most likely related to the water source. Tissue culture quality water is used, but different results are obtained from different sources.

   Vortex vigorously for 30 seconds (after the 30 min).

   Remove medium from cells and add 10 ml fresh serum containing medium. **THIS SHOULD CONTAIN 10 mM ammonium chloride.**

4. Add the Ca_2PO_4-DNA solution to the cells. Tilt the dish so that the media is at one end. Dropwise add 1 ml Ca_2PO_4-DNA solution per 10 ml media. Mix gently, but well before retuning the flask to its horizontal position. Incubate 4 hours.

   Note: Ca_2PO_4 lowers the pH, so it is added in this manner in order to avoid a pH shock to the cells.

5. Glycerol shock. This facilitates uptake of the DNA

   1) Aspirate the media.
   2) Add 3 ml of 15% glycerol in 1xPBS at one end of tilted dish
   3) Let the solution gently roll over the cells.
   4) After 3 min, aspirate the glycerol.
   5) Quickly wash the cells 2 times with 10 ml 1xPBS (add at one end of the dish).
   6) Add fresh media.
   7) Incubate the cells 48-72 hours before adding selection agent

Solutions:

2xHBS (Hepes Buffered Saline)

per liter		
Hepes	10 g, pH 7.05	Filter sterilize, aliquot.
NaCl	16 g	Store at -70°C.
KC1	0.74 g	Use only once.
Na_2HPO_4	0.40 g	
Dextrose	2 g	

10xPBS (Phosphate Buffered Saline)

per liter	
KC1	2.0 g
KH_2PO_4	2.0 g
NaCl	80 g
Na_2HPO_4	21.6 g

2M $CaCl_2$ — Autoclave. Aliquot. Store at -20°C