ProFection® Mammalian Transfection System

INSTRUCTIONS FOR USE OF PRODUCT E1200.

Quick PROTOCOL

Calcium-Phosphate Transfection Protocol

1. Plate cells one day before the transfection. A general guideline for plating density is 8×10^5 cells per 100mm plate.
2. Three hours before the transfection, replace cell culture medium with fresh growth medium.
3. Thaw all transfection reagents. Warm to room temperature and mix thoroughly.
4. For each transfection, prepare two tubes. Add DNA and water, followed by $CaCl_2$, to Tube 1. Add 2X HBS to Tube 2 (see table below).

	Per 60mm Plate	Per 100mm Plate
Tube 1		
DNA	6–12µg	10–20µg
2M $CaCl_2$	37µl	62µl
sterile, deionized water to a final volume of	300µl	500µl
Tube 2		
2X HBS	300µl	500µl

5. In a tissue culture hood, gently vortex the 2X HBS. Slowly add the DNA solution in Tube 1 dropwise to the HBS in Tube 2 while vortexing.
6. Incubate the combined solution at room temperature for 30 minutes.
7. Vortex again, then immediately add the solution, dropwise, to cells. Swirl plate to distribute. Incubate at 37°C with CO_2 for up to 48–72 hours.
8. After incubation cells may be harvested or treated with selective medium.

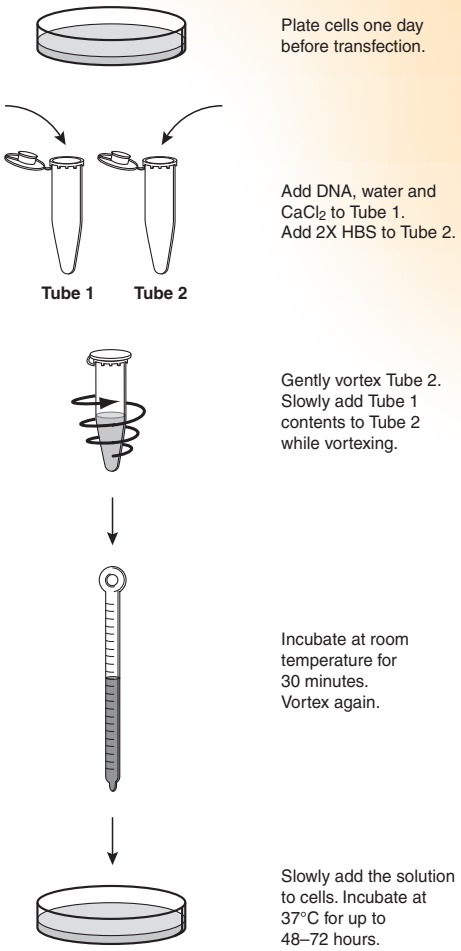

Plate cells one day before transfection.

Add DNA, water and $CaCl_2$ to Tube 1. Add 2X HBS to Tube 2.

Gently vortex Tube 2. Slowly add Tube 1 contents to Tube 2 while vortexing.

Incubate at room temperature for 30 minutes. Vortex again.

Slowly add the solution to cells. Incubate at 37°C for up to 48–72 hours.

For additional protocol information, see Technical Manual #TM012, available online at: **www.promega.com/tbs**

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