



Judd Rice Laboratory

at the USC/Norris Comprehensive Cancer Center

www.histonecode.com

Transient Transfection by Calcium Phosphate

Revised by C Pham
January 2007

Procedure

Volumes based upon one 6-well plate, 3mL total media volume. Filter solutions and store at 4°C.

1. Grow cell until they reach 80-90% confluence.
2. One hour before transfection, change the media.
3. Dilute 4ugs of DNA in water to have the final volume of 330ul in a 0.5ml tube.
4. Add 47 ul of 2M calcium chloride dropwise into the DNA tube. Tap gently to mix.
5. Within 1-2 min after the addition of CaCl₂ into the DNA tube, add 379ul of 2X HeBS dropwise. Tap the tube gently while adding the HeBS.
6. Add the DNA mixture dropwise into the cell well evenly. Move the plate back and forth to mix the DNA into the media.
7. After 7-11 hours, change the media.
8. Harvest the cells after 48 hours after transfection.

Solutions

2X HeBS

NaCl (0.28M final)

HEPES (.05M final)

sodium phosphate(1.5mM final)

- pH the solution with 5N NaOH between 7.05-7.12
- Check the pH before use.
- Use with 1-2 days for better transfection.