

LOW CO₂ TRANSFECTION

Materials:

2 x BBS

50 mM BES (pH6.96) N-N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid

280 mM NaCl

1.5 mM Na₂ HPO₄

pH adjust at room temp

Filter sterilize, store at -20°C

2.5 M CaCl₂ (sterile)

dH₂O (sterile)

Procedure:

For one transfection:

1. Trypsinize exponentially growing cells, seed 5x10⁵ cells/10 cm plate, and incubate overnight in 10 ml of growth medium.
2. Precipitate 20-30 µg of plasmid DNA. Spin. In hood, rinse pellet and tube with cold absolute ethanol. Remove ethanol and allow pellet to dry.
3. Resuspend pellet in 450 µl of sterile dH₂O.
Add 50 µl of 2.5M CaCl₂. Mix.
4. Add 0.5 ml of 2xBBS.
Incubate at room temperature for 10-20 min.
(Solution will become slightly cloudy).
5. Break up any clumps that might have formed with a 5 ml pipet, and add this 1 ml of CaPO₄ DNA solution dropwise to a plate of cells, swirling gently to mix well, and incubate for 15-24 hr at 35°C in 2-4% CO₂.
6. Remove the medium, rinse cells twice with growth medium, refeed, and incubate for 24 hr at 35-37°C in 5% CO₂.
7. Split cells at an appropriate ratio (>1:10) and incubate for an additional 24 hr before starting selection of stable transformants.
For transient transfections, harvest after the additional 24 hr. period.

Reference: *Chen, C. and Okayama H.*

