

Calcium Phosphate-Mediated Transfection of Primary Cultured Neuron

by Qiang Wang

Culture

We use low-density culture of hippocampal neurons on 18 mm coverslips which is supported by a layer of astrocytes on 60 mm dishes. Usually 3-10 days in vitro is good for transfection. According to experience and reference, for unknown reasons, it is hard to detect any expression for neurons younger than 3 days (sometimes you may get some). And neurons tend to be more fragile after 10 days.

Reagents:

DNA

Double CsCl gradients banding purification preferred (Qiagen kits usually don't work as well).

2×HeBS (Hepes Buffered Solution)

NaCl	274 mM
KCl	10 mM
Na ₂ HPO ₄ ·7H ₂ O	1.4 mM
D-glucose	15 mM
HEPES (free acid)	42 mM

Adjust pH with 10 N NaOH to pH 7.05-7.15. It is best to prepare several batches of different pH to test the best working condition. Once get the best batch, store in aliquots at -20°C. Filter sterilized.

2.5 M CaCl₂ Filter sterile.

Transfection Media

DMEM Gibco/BRL no. 11960 (pre-incubate in CO₂ incubator before transfection)

Procedure

The volume introduced here should be enough for 2-3 60 mm dishes with 5 18 mm coverslips each.

1. Put 150 ul HeBS in a tube.
2. Mix reagents in hood in a fresh tube:
 - 15 ul 2.5 M CaCl₂
 - 5-20 ug DNA
 - dH₂O to 150 ul total

3. Add DNA CaCl₂ mixture into HeBS, drop by drop, make sure swirling between each drop to mix well of each drop. This is critical for the formation of precipitation.
4. Place neuron to be transfected with cell side up in a fresh 60 mm dish with 3 ml of DMEM as transfection medium (pre-incubated in CO₂ incubator).
5. Drip DNA/ CaCl₂/HeBS in small drops over coverslips and incubate for 15-75 min. Find out a time point best for your condition. Check the forming of fine precipitation under a microscope periodically. Too much precipitation will lead to toxicity and too little precipitation result in low transfection efficiency.
6. Stop transfection by aspire the transfection medium and wash once with transfection medium. Transfer coverslips back to conditioned medium with glia layer.

100 uM APV can help survival of hippocampal neurons after transfection. The transfection efficiency varies. The protocol also can be modified and apply to different neurons.