

Calciumfectin™ Mammalian Transfection Kit (Cat. # G099)

This kit provides high-quality reagents suitable for both transient and stable transfections. The kit includes enough materials to perform 100 transfections in 10-cm plates.

I. List of Components

Store HBS buffer at -20°C ; all other components at 4°C .

- 6 ml 2 M Calcium Solution
- 1 x 50 ml 2X HEPES-Buffered Saline (HBS)

We recommend dispensing this buffer into small aliquots, to be stored at -20°C . Avoid multiple freeze-thaw cycles. When an aliquot is in use, store it at 4°C for up to one week.

- 1 x 70 ml Sterile H₂O

II. Calciumfectin™ Mammalian Transfection Protocol

The following protocol is applicable to adherent cultures growing in 100-mm tissue-culture plates. If you are using other types of culture vessels, adjust the volume of the transfection solution with information provided in the table.

1. Plate the cells the day before the transfection experiment at 50–80% density one day before transfection.
2. 0.5–3 hrs prior to transfection, replace culture medium with 10 ml of fresh medium.
3. For each transfection, prepare Solution A and Solution B in separate sterile tubes.

Solution A: add components in the following order:

- 10–20 μg Plasmid DNA
- X μl Sterile H₂O
- 64 μl 2 M Calcium Solution
- 500 μl Total Volume

Solution B: 500 μl 2X HBS

4. Carefully and slowly vortex Solution B while adding Solution A dropwise. (Alternatively, blow bubbles into Solution B with a 1-ml sterile pipette and an autopipettor while adding Solution A dropwise.)
5. Incubate the transfection solution at room temperature for 20 min.

II. Calciumfectin™ Mammalian Transfection Protocol (continued)

6. Gently vortex transfection solution and then add solution drop-wise to culture medium uniformly.
7. Incubate at 37°C for 5–7 hrs in a CO₂ incubator.
9. Remove calcium phosphate-containing medium and feed cells with 10ml complete growth medium and incubate at 37°C until needed for assay.
11. Assay for transient gene expression or start selection for stable transformants 24–72 hr post-transfection.

Culture Plate Conversions

| Size of Plate | Growth Area | Relative Area*(cm ²) | Recommended Volume |
|---------------|-------------|----------------------------------|--------------------|
| 96 well | 0.32 | 0.04 X | 200 µl |
| 24 well | 1.88 | 0.25 X | 500 µl |
| 12 well | 3.83 | 0.5 X | 1.0 ml |
| 6 well | 9.4 | 1.2 X | 2.0 ml |
| 35 mm* | 8.0 | 1.0X | 2.0 ml |
| 60 mm | 21 | 2.6 X | 5.0 ml |
| 10 cm | 55 | 7 X | 10.0 ml |
| Flasks | 25 | 3 X | 5.0 ml |
| | 75 | 9 X | 12.0 ml |

* Relative area is expressed as a factor of the growth area of a 35-mm culture plate.