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Calciumfectin™ Mammalian Transfection Kit

Store at 4°C

Cat. No.	Description	Quantity
G099	Calciumfectin™	Kit (100 transfections)

Description
The Calciumfectin™ Mammalian Transfection Kit provides high-quality reagents suitable for both transient and stable transfections. This kit includes a sufficient volume to perform 100 transfections when using 10 cm plates.

List of Components
Store HBS buffer at -20°C and 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 only.
- 1 x 70 ml sterile H_2O

Protocol
The following protocol is applicable to adherent cultures growing in 10 cm tissue-culture plates. If you are using other types of culture vessels, adjust the volume of the transfection solution as needed, by referring to the information provided in Table 1 as a guide.

1. Plate the cells one day before the transfection at 50-80% density.
2. Between 0.5 - 3 hrs before the 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 ul Sterile H_2O
   64 µl 2 M Calcium Solution
   = 500 µl Total Volume
   Solution B: 500 µl 2X HBS

4. Gently pulse vortex Solution B while adding Solution A in a dropwise manner. (Alternatively, blow bubbles into Solution B with a 1 ml sterile pipette and an autopipettor while adding Solution A in a dropwise manner).

5. Incubate the transfection solution at room temperature for 20 mins.
6. Gently vortex the transfection solution once more, then add the solution in drops to the culture medium in a uniform manner so it is evenly distributed.
7. Incubate at 37°C for 5-7 hrs in a CO_2 incubator.
8. Remove the calcium phosphate-containing medium and feed the cells with 10 ml complete growth medium. Incubate at 37°C until needed for downstream assays.
9. Assay for transient gene expression or start selection for stable transformants 24-72 hrs post-transfection.

Table 1: 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
35mm*	**8.0**	**1.0X**	**2.0 ml**
60mm	21	2.6 X	5.0 ml
10cm	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.