

Calcium Phosphate Transfection Kit (TBS8201, Store at 4°C)

Catalog	Tests for 10 cm plate
TBS8201-40	40
TBS8201-120	120

Product description

Calcium phosphate transfection is a powerful method to introduce DNA plasmid into eukaryotic cells. The principle is to mix phosphate buffer with CaCl_2 solution containing DNA plasmid to form DNA-calcium phosphate precipitate. This precipitate can adhere to the cell and eventually transfer DNA into the target cell by endocytosis.

Tribioscience Calcium Phosphate Transfection kit provides high-quality pretested reagents suitable for cell transfection needs. The kit includes all the reagents necessary to perform cell transfection.

Synonyms: Calcium Transfection, Gene Delivery, Mammalian Transfection,

Main features

- **Accurate:** Optimized conditions for highly consistent transfections.
- **High efficiency:** High transfection efficiency in many types of mammalian cells.
- **Flexible:** Multiple size choices for different needs.

Kit composition, tests and storage conditions

Components	TBS8201-40	TBS8201-120
2.5M CaCl_2	3.2 mL	9.6 mL
2x HBS	32 mL	96 mL
Sterile Water	32 mL	32 mL

Note: Store the components at 4 °C for shelf-life of 2 years.

Experimental operation

The following protocol is designed for use with adherent cultures growing in a well of a 6-well plate. If you prepare different sizes of plates, wells, or flask, please calculate the buffer volume according to Table 1 as a reference.

Day 1: Prepare cultured cells for transfection

Seed cells in 6-well plate, cultured in a 5% CO_2 incubator at 37°C for 18-24 hours. The cell should be

50-80% confluent on the day of transfection. Generally, the cell number is about $2-4 \times 10^5$ / well of a 6- well plate.

Day 2: Transfection

1. 30-60min before transfection, replace 2 mL fresh culture medium.
2. Warm up the reagents required for transfection to 37°C in water bath or dry bath.
3. Prepare transfection mixture as below:

Transfection Mix Preparation

DNA- Calcium Complex	Volume (μl)
Plasmid DNA (1-3ug)	XX
Sterile water	90 - XX
2.5M Calcium Solution	10
Final volume (ul)	100

4. Add 100 μl of 2x HBS dropwise into above DNA-Calcium complex, mixing gently to form DNA- CaCl_2 -HBS transfection solution.
5. Incubate the transfection solution at room temperature for 20 min.
6. Gently vortex transfection solution and then add the solution dropwise to culture plate medium (Add 200 μl) of transfection solution per well of a 6-well plate)
7. Gently move plates back and forth to distribute transfection solution evenly (Note: Do not rotate the plates as this will concentrate transfection precipitate in the center of the well or plate).
8. Incubate plate at 37°C for overnight in CO_2 incubator.

Day 3: Change media

9. Remove the media from the cells.
10. Wash the cells with medium or 1x PBS.
11. Add 2 mL fresh complete growth medium and incubate the cell at 37°C until needed for assay.

Day 4-5: Expression analysis

12. Expression Analysis: Assay for transient gene expression or start selection for stable transformants at 24-72 hours post-transfection.

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Table 1: Plate size, DNA amount and Buffer Size Conversions

	DNA-Calcium Complex				
Plate/ Flask	Plasmid DNA	2.5M Calcium Solution	Sterile H ₂ O	2X HBS	Culture Medium
96-well	30–100 ng	0.4 µl	to 4 µl total	4 µl	200 µl
24-well	200–600 ng	2.5 µl	to 25 µl total	25 µl	500 µl
12-well	0.4–1.2 µg	5 µl	to 50 µl total	50 µl	1 mL
6-well	1–3 µg	10 µl	to 100 µl total	100 µl	2 mL
35 mm	1–3 µg	10 µl	to 100 µl total	100 µl	2 mL
60 mm	2.5–7.5 µg	26 µl	to 260 µl total	260 µl	5 mL
10 cm	6–18 µg	70 µl	to 700 µl total	700 µl	10 mL
T25 flask	3–9 µg	30 µl	to 300 µl total	300 µl	5 mL
T75 flask	8–24 µg	90 µl	to 900 µl total	900 µl	12 mL

Relative products:

1.25M Calcium Solution (TBS5071)

2.5 M Calcium Solution (TBS5072)

2x HBS Buffer (TBS5076)

DMEM Medium (TBS8061)

RPMI-1640 Medium (TBS8063)

MSC Medium (TBS8021)

Hams F-12 Medium (TBS8032)

ESC/iPSC-qualified FBS (TBS8002)

DMEM/F-12 Medium (TBS8083)

0.1% Gelatin Solution (TBS8004)

1xPBS (TBS5003)

Research use only