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Chemistry beyond the ordinary

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Transporter™ 5 Transfection Reagent

OVERVIEW:

Transporter™ 5 transfection reagent is the ready-to-use form of a proprietary linear polyethylenimine (PEI) derivative. Transporter™ 5 condenses DNA into positively charged complexes which readily enter the cell by endocytosis. The Transporter™ 5-DNA complex is highly resistant to endosomal degradation, allowing high transfection efficiency.

Transporter™ 5 transfection reagent effectively introduces DNA into HEK-293, CHO, COS, HeLa, insect (Sf9 and Sf21) and other eukaryotic cell lines. Transporter™ 5 is most compatible with plasmids up to 135,000 nucleotides in size, although larger plasmids can be used with some success.

REAGENTS INCLUDED:

- Transporter™ 5 transfection reagent: 1-5 x 1 mL vial of reagent containing 1 µg/µL proprietary linear PEI derivative. 1 mL is sufficient for transfecting a total of 250 µg of DNA, or about 60-120 transfections in 6 well plates.

REAGENTS TO PREPARE:

- Diluent: A 150 mM NaCl solution prepared with sterile, WFI/Cell Culture Water. A solution of 25 mL (containing 0.2912 g NaCl) is sufficient for 1 mL of Transporter™ 5 transfection reagent.

PROTOCOL FOR ADHERENT CELLS:

Preparation:

- Plate cells 18 to 24 hours before transfection.
- Use an appropriate number of cells in seeding solution to obtain a cell monolayer with 60-80% confluence. These qualities will provide the most optimal conditions for transfection. See Table 1 for suggested guidelines.
- Note: High serum levels inhibit the efficacy of Transporter 5™. In most cases, low serum levels (≤ 5%) will produce the highest transfection efficiency.

Table 1- Guidelines for seeding adherent cell culture vessels.

Culture Vessel	Total Surface area (cm^2)	Cells in seeding solution
96-well plate	0.3	$[1.2 – 2.4] \times 10^4$
48-well plate	1.0	$[4.0 – 8.0] \times 10^4$
24-well plate	1.9	$[0.8 – 1.6] \times 10^5$
12-well plate	3.5	$[1.5 – 3.0] \times 10^5$
6-well plate	9.6	$[4.0 – 8.0] \times 10^5$
35 mm dish	9.6	$[3.5 – 7.0] \times 10^5$
60 mm dish	21	$[0.9 – 1.8] \times 10^6$
100 mm dish	58	$[2.2 – 4.4] \times 10^6$
T75 flask	75	$[3.0 – 6.0] \times 10^6$
T175 flask	175	$[0.7 – 1.4] \times 10^7$

Sample Transfection Protocol (Single well in 6-well plate):

1. 1 to 2 hours before transfection, exchange growth media in single well with 3 mL fresh growth media containing 2% serum.
2. Prepare Transporter™ 5-DNA transfection mixture (order is critical):
    i. To 300 µL diluent in polypropylene tube add 2 µg plasmid DNA.
    ii. Briefly mix/vortex solution.
    iii. Add 8 µL Transporter™ 5 to mixture. (1:4 DNA/Transporter)
    iv. Vortex for 5 seconds.
    v. Let solution sit for 20 minutes in hooded environment to allow Transporter™ 5-DNA complexes to form.
    vi. Mix solution gently by pipetting up and down 3 times.
3. Add Transporter™ 5-DNA transfection mixture to well.

Note: The preceding protocol can easily be scaled up or down by adjusting the volume of the Transporter™ 5-DNA transfection mixture in diluent to 10% of the overall culture volume. Ensure that the DNA/Transporter™ 5 ratio is 1:4. See Table 2 for recommended amounts of reagents for various culture vessels.

Incubation

- Following addition of Transporter™ 5-DNA transfection mixture to well, return wells to incubator.
- Typically, recombinant protein is detectable at 36-48 hours after transfection. Maximal expression is usually observed 72-96 hours after transfection.

Table 2 - Reagent quantities for transfection in a variety of culture vessels.

Culture Vessel	Culture Volume (mL)	Plasmid DNA (µg)	Diluent (mL)	Transporter™ 5 (µL)
6-well plate, single well	3	2 – 4	0.3	8 – 16
35 mm dish	3	2 – 4	0.3	8 – 16
60 mm dish	5	6 – 12	0.5	24 – 48
100 mm dish	10	12 – 24	1.0	48 – 96
T75 flask	15	18 – 36	1.5	72 – 144
250 mL shake flask	50	50 – 100	2.5	200 – 400

PROTOCOL FOR SUSPENSION CELLS:

Preparation:

- 2 to 3 hours before transfection, seed cells at 1.0 x 10^6 per mL of culture.

Sample Transfection Protocol (50 mL culture in 250 mL shake flask)

1. Prepare Transporter™ 5-DNA transfection mixture (order is critical):
    i. To 2.5 mL of diluent in polypropylene tube, add 50 µg of plasmid DNA.
    ii. Briefly mix/vortex solution.
    iii. Add 200 µL of Transporter™ 5 to mixture.
    iv. Vortex solution for 5 seconds.
    v. Let solution sit for 20 minutes in hooded environment to allow Transporter™ 5-DNA complexes to form.
    vi. Gently mix solution by pipetting up and down 3 times.

2. Add entire transfection solution to 25 mL of cell suspension culture.
3. Return cell suspension to incubator.

Incubation

- Shake for 2 to 3 hours in incubator, then add 25 mL of fresh culture medium. Return to incubator.
- Typically, recombinant protein is detectable at 36-48 hours after transfection. Maximal expression is usually observed 72-96 hours after transfection.

ORDERING INFORMATION

Catalog No.	Description	Size(s)
26008	Transporter™ 5 Transfection Reagent	(1 mL), 26008-5 (5 x 1 mL)

RELATED PRODUCTS

26253	Cell Culture Water	500 mL, 1000 mL, 6 x 500 mL, 6 x 1000 mL
25626	Cell Culture Bag, 50 mL with Filment	15 bags per box
25628	Cell Culture Bag, 250 mL with Filment	10 bags per box
25631	Cell Culture Bag, 1 L with Filment	5 bags per box
25634	Cell Culture Bag, 2 L with Filment	5 bags per box
25627	Cell Culture Bag, 50 mL with 2 Ports	15 bags per box
25630	Cell Culture Bag, 250 mL with 2 Ports	10 bags per box
25633	Cell Culture Bag, 1 L with 3 Ports	5 bags per box
25636	Cell Culture Bag, 2 L with 3 Ports	5 bags per box
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