

EntransterTM-H Transfection Reagent

For the transient and stable transfection of HEK 293T/Hela/CHO cells

Cat. No. 18668-04 Size: 0.4ml
Store at +4°C

Description

Engreen Biosystem Co,Ltd. is a professional R&D manufacturer of transfection reagents. EntransterTM-H is a nano-polymer transfection reagent, synthesized by Engreen Biosystem Co. Ltd.

EntransterTM-H is a patent-pending, animal-origin free formulation for the transfection of DNA into HEK 293T/Hela/CHO cells providing the following advantages:

- 80-98% transfection efficiency in HEK 293T/Hela/CHO cells, which is reproducible with low toxicity.
- Save DNA. For example, ONLY 10% usual DNA amount transfected into HEK 293T cells can get 98% transfection efficiency.
- EntransterTM-H complexes can be added directly to cells in culture medium, in the presence or absence of antibiotics and serum.
- It is not necessary to remove complexes or change/add medium after transfection until next necessary medium change.

Important Guidelines for Transfection

- Antibiotics DO NOT affect transfection efficiency or product toxicity during the EntransterTM-H transfection procedure.
- It is NOT necessary to change the medium after transfection 4-6 hours, medium can be replaced after 18 hours.
- Due to the low toxicity of EntransterTM-H, cells can be **40-70% confluent** at the time of transfection. This can offer cells more culture time (e.g. 48-72 hours) after transfection, and obtain sufficient biological effects.
- Optimizing transfection. To obtain the highest transfection efficiency and lowest non-specific effects, optimize transfection conditions by varying DNA amount and EntransterTM-H concentrations. Due to some factors, different optimization conditions in different labs have been observed. Various optimizations may be required to obtain maximal transfection efficiency and protein expression with minimal cytotoxicity. The main parameters optimized

with this protocol are in Table 1. Once high-efficiency conditions of your lab have been established, the following transfection experiments will be stabilized.

Table 1. Volumes of Entranster™-H solution and amounts of DNA for various ratios (6-well/35mm).

	HEK 293T cell			Hela, CHO cell			
	1	2	3	1	2	3	4
DNA	0.5µg	1µg	2µg	1µg	1µg	2µg	2µg
Entranster™-H	0.5µl	1µl	2µl	1µl	2µl	2µl	4µl

Transfection Procedure

Use the following procedure to transfect cells in a 35-mm culture dish format. For other formats, see **Scaling Up or Down Transfections**. All amounts and volumes are given on a per well basis.

1. Preparation of cells for transfection.

One day before the transfection experiment, trypsinize, adjust the cell concentration, and plate the cells in the chosen cell-culture vessel. Plating $0.8-3 \times 10^5$ cells in a 35-mm culture dish in 2 ml of medium (or a six-well plate) overnight will achieve the desired density of 40–70% confluency. If using culture plates of a different size, adjust the starting volume of Entranster™-H Reagent and the starting mass of DNA in proportion to the relative surface area (Table 2).

2. Preparation of Entranster™-H Reagent:DNA complex and transfection of cells

- a. Dilute DNA in 250 µl of serum-free medium (e.g. Opti-MEM® I Medium, PBS or 150mM NaCl). Mix gently.
 - b. Mix Entranster™-H gently before use, then dilute the appropriate amount in 250 µl of serum-free medium (e.g. Opti-MEM® I Medium, PBS or 150mM NaCl). Incubate for 5 minutes at room temperature.
 - c. After 5 minutes incubation, combine the diluted DNA with diluted Entranster™-H (total volume = 500 µl). Mix gently and incubate for 15 minutes at room temperature. Note: Complexes are stable for 6 hours at room temperature.
3. Add the 500 µl of complexes to each well containing cells and medium in the presence or absence of antibiotics and serum. Mix gently by rocking the plate back and forth.
 4. Incubate cells at 37°C in a CO₂ incubator for 36-48 hours prior to testing for transgene expression. It is not necessary to change the medium after transfection 4-6 hours, medium can be replaced after 18 hours.
 5. For stable cell lines: Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours after transfection. Add selective medium (if desired) the following day. For suspension cells: 4 hours post-transfection, add PMA and/or PHA (if desired) to enhance CMV promoter activity and increase gene expression.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Entranster™-H, DNA, cells, and medium used in proportion to the relative surface area, as shown in the table 2. With automated and high-throughput systems, a complexing volume of 50 µl is recommended for transfections in 96-well plates. Note: You may perform rapid 96-well plate transfections by plating cells directly into the transfection mixture. Prepare complexes in the plate and directly add cells at twice as the cell density as in the basic protocol in a 100 µl volume. Cells will adhere as usual in the presence of complexes.

Table 2 Reagents amount for different incubation containers

Culture vessel	Surface Area per well (cm ²)	Ratio of Surface Area to 24-well	Vol. of plating medium	DNA (µg) in media vol. (µl)	Entranster™-H (µl) in media vol. (µl)
96-well	0.3	0.2	0.1ml	0.02-0.08µg in 25 µl	0.02-0.16µl in 25 µl
24-well	1.9	1	0.5ml	0.04-0.16µg in 50 µl	0.04-0.32µl in 50 µl
12-well	3.8	2	1ml	0.1-0.4µg in 100 µl	0.1-0.8µl in 100 µl
6-well/35-mm	10	5	2ml	0.5-2µg in 250 µl	0.5-4µl in 250 µl
60 mm/T25 flask	21	10	50ml	1-4µg in 500 µl	1-8µl in 500 µl
100 mm/T75 flask	58	30	15ml	3-12µg in 1.5 ml	3-24µl in 1.5 ml

Storage and stability

- Entranster™-H Reagent is shipped at room temperature.
- Entranster™-H Transfection Reagent is stabilized for extended storage at +2 to +8°C through the expiration date printed on the label (one year from the date of manufacture) when very tightly closed.

Quality control

- Functional analysis
Two microliters of Entranster™-H Transfection Reagent is combined with 2 µg of reporter-gene vector DNA, and used to transfect Hela cells (in a monolayer [50–70% confluent]) in the presence of 10% fetal bovine serum (FBS). Following transfection, the percentage of transfected cells is analyzed. Typically, 50–80% of Hela cells express reporter-gene protein.
- Cytotoxicity analysis
Hela cells that are continuously exposed to Entranster™-H Reagent for 26 hours, with or without DNA, in the presence of serum, and without a change of medium, are >90% viable by flow-cytometric analysis based on propidium-iodide staining.

Related products

Enfactor™: For efficient DNA transfection of a broad range of cell lines.

Entranster™-R: Transfect siRNA into animal cells.

Entranster™-in vivo: In vivo transfection.

Notice to Purchaser

Purchaser represents and warrants that Entranster™-H Transfection Reagent will be used only for research purposes. Transfected cells, materials produced and any data derived from the use of Entranster™-H Transfection Reagent, should be used only for the internal research of Purchaser whether Purchaser is a “for-profit” or a “non-profit” organization. Under no circumstances may Entranster™-H Transfection Reagent be used by Purchaser or any third party for a commercial purpose unless Purchaser has negotiated a license for commercial use with Engreen Biosystem (contact information: License@Engreen.com.cn). For purposes of the foregoing sentence, “commercial purpose” shall mean use of Entranster™-H Transfection Reagent for profit or commercial gain. By using Entranster™-H Transfection Reagent, Purchaser agrees to be bound by the above terms. If Purchaser wishes not to be bound by these terms, Purchaser agrees to return the Entranster™-H Transfection Reagent to Engreen Biosystem for a full refund.

Trademarks

Entranster and Enfactor are registered trademarks of Engreen Biosystem Co.Ltd,China. Opti-MEM is a registered trademark of Invitrogen Corporation. Other brands or product names are trademarks of their respective holders.

Contact and Support

To ask questions, solve problems, suggest enhancements, or report new applications, please visit our Online Technical Support Site at: www.engreen.com.cn/support.htm
To call, write, fax, or e-mail us, visit the Engreen Biosystem home page, www.engreen.com.cn, and contact the distributor in your home country. Utilize the Product Search function to access Pack Inserts and Material Safety Data Sheets.

© 2008 Engreen Biosystem. All rights reserved.