HighGene plus Transfection reagent

Catalog: RM09014P

Size: 1000μL/10000μL

Description

HighGene plus is a proprietary formulation for the transfection of nucleic acids (DNA and RNA) into eukaryotic cells providing the following advantages:

·Highest transfection efficiency in many cell types and formats (e.g. 96-well).

· HighGene plus complexes can be added directly to cells in culture medium (including serum-free media), in the presence or absence of serum and antibiotics.

·It is not necessary to prepare the specific reduced serum medium to dilute HighGene plus and DNA before complexing.

Storage Conditions:

·Store at -20℃.

Required Materials:

·Plasmid DNA (0.5-2 μg/μL stock)

·Tubes

Important Guidelines for Transfection

·Prepare complexes using the DNA (μg) to HighGene plus (μL) ratio of 1:1 or 1:2 for most cell

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lines. Optimization may be necessary.

· Transfect cells at high cell density: 85-95% confluence at the time of transfection is recommended for high efficiency, high expression levels, and to minimize cytotoxicity. Optimization may be necessary. Maintain the same seeding conditions between experiments.

· Incubation period of transfection complexes in cells: From at least 6 hours to at most 24 hours. Optimization may be necessary.

Transfection Procedure (for DNA)

Use the following procedure to transfect mammalian cells in a 6-well format. For other formats, see Scaling Up or Down Transfections. All amounts and volumes are given on a per well basis.

1. Day 1: Seed cells:

Adherent cells: plate 2-5×10^5 cells in 2 mL of growth medium so that cells will be 90-95% confluent at the time of transfection.

Suspension cells: Just prior to preparing complexes, plate 4-8×10^5 cells in 2 mL of growth medium.

2. Day 2: Prepare transfection complexes and transfection procedure

For each transfection sample, prepare complexes as follows:

a. Dilute DNA in 250 µL of medium without serum. Mix gently.

b. Mix HighGene plus gently before use, then dilute the appropriate amount in 250 µL of medium without serum. Incubate for 5 minutes at room temperature.

Note: Combine diluted DNA with diluted HighGene plus within 30 minutes.

c. After 5 minute incubation, combine diluted DNA with diluted HighGene plus (total volume = 500 μL). Mix gently and incubate for 20-30 minutes at room temperature.

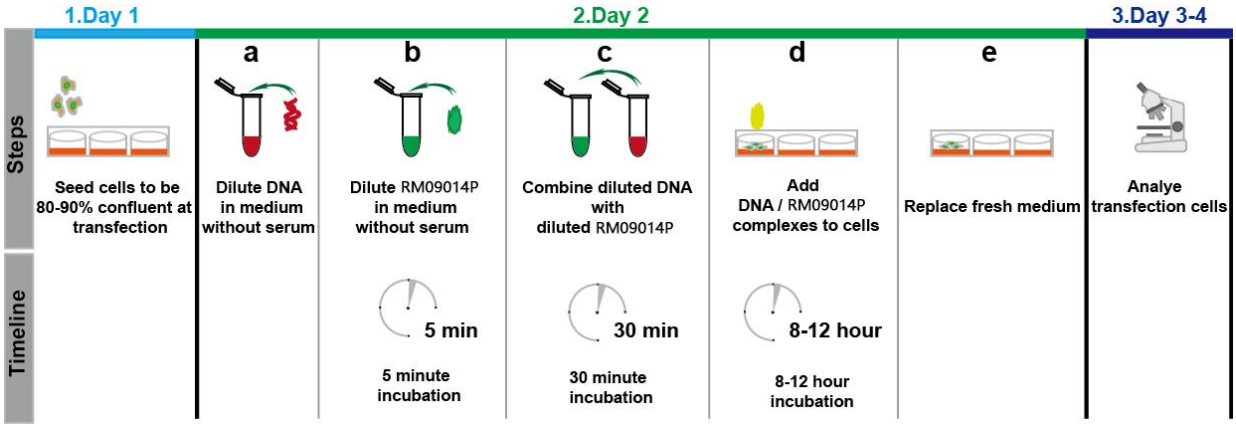
Note: Complexes are stable for 6 hours at room temperature.

d. Add the 500 μL of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.

e. Incubate cells at 37°C in a CO_2 incubator for 8-12 hours prior to replace 2 mL of fresh growth medium,

3. Day3-4: Incubate cells for 18-48 hours to testing for transgene expression.

Schematic diagram of transfection steps



Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of HighGene plus, DNA, cells, and medium used in proportion to the relative surface area, as shown in the table.

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Culture vessel	Surf. area per well	Vol. of plating medium	DNA (μg) in media vol. (μl)	RM09014P (μl) in media vol. (μl)
96-well	0.3 cm^2	100 ul	0.2 μg in 25 μl	0.2 μl in 25 μl
24-well	2 cm^2	500 ul	0.8 μg in 50 μl	0.8 μl in 50 μl
12-well	4 cm^2	1 ml	1.6 μg in 100 μl	1.6 μl in 100 μl
35-mm	10 cm^2	2 ml	4.0 μg in 250 μl	4.0 μl in 250 μl
6-well	10 cm^2	2 ml	4.0 μg in 250 μl	4.0 μl in 250 μl
60-mm	20 cm^2	5 ml	8.0 μg in 250 μl	8.0 μl in 250 μl
10-cm	60 cm^2	10 ml	24 μg in 250 μl	24 μl in 250 μl

Note: Surface areas are determined from actual measurements of tissue culture vessels, and may vary depending on the manufacturer.

Optimizing Transfection

To obtain the highest transfection efficiency and low cytotoxic effects, optimize transfection conditions by varying cell density, DNA and HighGene plus concentrations as well as incubation period of transfection complexes in cells. Make sure that cells are greater than 85% confluent, vary DNA (μg): HighGene plus (μL) ratios of 1:1 or 1:2 and vary incubation period of transfection complexes in cells from at least 6 hours to at most 24 hours.

Quality Control

HighGene plus is tested for the absence of microbial contamination using blood agar plates, sabaraud dextrose agar plates, and fluid thioglycolate medium, and functionally by transfection of HEK-293T cells with an EGFP reporter plasmid.

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