Protocol ● EndoFectin™ HepG2 Transfection Reagent ● Catalog Nos. EF005/006

For efficient transfection of nucleic acids into HepG2 hepatocellular carcinoma cells

Description

EndoFectin™ HepG2 Transfection Reagent is a proprietary lipid-based formulation that forms a complex with nucleic acids and transports the complex into animal cells. EndoFectin™ HepG2 has been proven to work efficiently in the hepatocellular carcinoma cell line HepG2. It is optimized for efficient and simple delivery of nucleic acids even in the presence of serum. EndoFectin™ HepG2 provides the following advantages:

- Superior transfection efficiency of HepG2 cells compared with commonly used transfection reagents, such as Lipofectamine® 2000
- Low cytotoxicity
- Does not require removal of serum or culture medium.
- Does not require washing or changing of medium after transfection
- For overexpression, knockdown, knockout, as well as high-throughput applications.

Contents and storage

Each vial contains 1 ml of sterile EndoFectin™ HepG2 reagent.

EndoFectin™ HepG2 is shipped at ambient temperature. Store the reagent at 4–8°C with the cap tightly closed. The reagent is stable for at least 12 months when stored at 4–8°C.

Quality control

Every lot of EndoFectin™ HepG2 is tested by transfecting subconfluent HepG2 cells with an eGFP-expressing plasmid (GeneCopoeia Catalog Number EX-EGFP-M02). Over 95% of cells expressed eGFP 16 hours post-transfection.

Before you start

Quality of plasmid

It is critical to use endotoxin-free plasmid DNA of the highest quality. Determine the DNA concentration by reading the absorption at 260 nm. DNA purity is measured by using the 260 nm / 280 nm ratio (the ratio should be in the range of 1.8 to 2.0). If possible, check the plasmid integrity by agarose gel electrophoresis.

Condition of cells

Always use high-quality cells that are well maintained and routinely authenticated which includes testing for bacteria, fungi, or Mycoplasma contamination. If the cells are from a recent liquid nitrogen stock, passage the cells at least 2 times before transfection.

Protocol for transient transfection

Materials:

- EndoFectin™ HepG2 transfection reagent
- Opti-MEM® I Reduced Serum Medium (Life Technologies**.** Catalog number: 31985-088).

1. Before beginning a transfection experiment, we recommend first optimizing your transfection conditions with the EndoFectin™ HepG2 transfection reagents. We suggest testing the amounts of EndoFectin™ HepG2 transfection reagent listed in Table 1.

Table 1. Suggested starting conditions for transfection of adherent cells.

Culture vessel	Surface area (cm^2)	Medium volume	Total amount of DNA per well	DNA dilution volume	EndoFectin volume per well	EndoFectin dilution volume
96-well plate (one well)	0.3	100 µl	100 ng	5 µl	0.2 µl	5 µl
24-well plate (one well)	1.9	0.5 ml	0.5 µg	25 µl	1 µl	25 µl
12-well plate (one well)	4.0	1.0 ml	1 µg	50 µl	2 µl	50 µl
6-well plate (one well)	9.3	2.0 ml	2.5 µg	125 µl	5 µl	125 µl
3.5-cm dish	7.5	2.0 ml	2.5 µg	125 µl	5 µl	125 µl
6-cm dish	21.0	5.0 ml	5 µg	250 µl	10 µl	250 µl
10-cm dish	49.0	10 ml	15 µg	750 µl	30 µl	750 µl

You can do the transfection directly in the preferred culture vessels listed in Table 1, or just do the test in a 96-well plate only. The optimized amount of EndoFectin™ HepG2 transfection reagent can be scaled up accordingly.

2. **T**he day before transfection, trypsinize and count the cells. Adjust the cell concentration and plate the cells in a cell-culture vessel with a total volume as suggested in Table 1. Do not include antibiotic. The number of cells plated in each well should be determined to be 90-95% confluent at the time of transfection.

3. Equilibrate DNA, EndoFectin™ HepG2 reagent, and Opti-MEM® I to room temperature

4. Dilute the required amount of DNA with Opti-MEM® I. Refer to Table 1 for suggested volumes.

5. Incubate the mixture for 5 minutes at room temperature. Once the transfection reagent is diluted, combine it with the DNA within 30 min.

6. Combine the diluted DNA with the diluted transfection reagent. Incubate at room temperature for 5 to 20 min to allow DNA-Transfection Reagent complexes to form.

7. Add the DNA- Transfection Reagent complexes directly to each well/dish and mix gently by rocking the plate back and forth. For scaling up, it is better to add the DNA- EndoFectin™ HepG2 complexes to the cells drop by drop into the culture medium (try to avoid adding directly to the cells).

8. Incubate the cells at 37°C in a CO_2 incubator for a total of 24-48 hours until they are ready to be assayed for transgene expression.