COSFectin™ Cell Line Specific Lipid

0.5 ml	170-3370
1.0 ml	170-3371
5 x 1.0 ml	170-3372

For Research Use Only
Store at 4°C

Storage and Stability

COSFectin lipid reagent is shipped on ice. Store at 4ºC upon receipt. Do not store below 0ºC. Invert the tube to mix contents before using.

Contents

COSFectin contains either 0.5 ml (catalog # 170-3370), 1.0 ml (catalog # 170-3371), or 5 x 1.0 ml (catalog # 170-3372) of a proprietary lipid formulation. One milliliter is generally sufficient for 200 cell transfections in 35 mm plates. COSFectin comes as a 1.0 mg total lipid/ml of solution containing sodium butyrate.

Overview

COSFectin lipid reagent is a mixture of a proprietary cationic compound and a co-lipid formulated in sodium butyrate solution. These compounds have been optimized specifically for high-efficiency transfection into cultured COS cells for overexpression of a desired protein. COSFectin is a very flexible lipid reagent that works well both in the presence and absence of serum at cell densities from 50 to 90%, with minimal cellular toxicity. Best results are typically obtained in the presence of serum at cell densities from 70 to 90% using the amounts of COSFectin and nucleic acid recommended in this manual.

Recommendations for Best Results

- In general, use a ratio of DNA (µg) to lipid (µl) of 1:1 to 2:1 to obtain the best transfection results.
- Invert the tube to mix contents before using.
- COSFectin works best in serum but can be used in serum-free medium.
- Use sterile polystyrene plasticware (e.g., 12 x 75 mm tubes or multiwell trays) to prepare the plasmid and lipid solutions. Polystyrene is recommended because cationic lipid-plasmid complexes may bind to polypropylene.

Optimization

Determining the optimum conditions for transfection efficiency is essential for maximal gene expression and to minimize cellular toxicity. The three most important parameters to optimize transfection efficiency are: amount of COSFectin, concentration of nucleic acid, and cell density. See Table 1 for suggested reagent and DNA concentrations for different culture vessel sizes.

Highest levels of expression can be obtained using concentrations of COSFectin and DNA suggested in this manual for given vessel sizes. If currently using a cationic lipid for transfection, start using COSFectin at current conditions and also try reducing and increasing the volume of COSFectin from 25 to 50% of current amount.

Table 1. Suggested Reagent Quantities for Different Sizes of Plates or Wells

Culture Vessel Size	Volume of Plating Medium	Plasmid DNA	Volume of Serum-Free Medium	COSFectin Reagent
96-well	0.1 ml	100–200 ng	20 µl	0.2–0.5 µl
24-well	0.5 ml	0.5–2.0 µg	50 µl	0.5–4.0 µl
12-well	1.0 ml	1.0–4.0 µg	100 µl	1.0–6.0 µl
6-well/35 mm	2.0 ml	2.0– 8.0 µg	250 µl	2.5–15 µl
60 mm	5.0 ml	5.0–20 µg	500 µl	5.0–30 µl
100 mm	10.0 ml	1.0–40 µg	1.0 ml	10–60 µl

Protocol for Transfection of Adherent COS Cells
(24-Well Plates)

1. One or two days before inoculating the cells for transfection, divide the culture so they will be actively growing (80 to 90% confluent) at the time of plating.
2. The day before transfection, inoculate 24-well plates with an appropriate number of cells in serum-containing medium such that the cells will be 70 to 90% confluent the following day. Plating 0.5 to 0.75 x 10^5 cells in 0.5 ml medium per well should give these confluences. Incubate the cells at 37°C in a 5% CO_2 incubator for 24 hr.
3. In 25 µl of serum-free medium, prepare nucleic acid to have a final concentration of 1.0 to 2.0 µg per well. (for different sized culture vessels, see recommended amounts in Table 1)
4. In a polystyrene container, prepare the COSFectin lipid reagent in 25 µl of serum-free medium.
   (for different sized culture vessels, see recommended volumes in Table 1)
5. Mix the nucleic acid-COSFectin solutions together by tapping or pipetting. Incubate for 20 minutes at room temperature, to allow complexes to form.
6. Add 50 µl of the complexes directly to the cells in serum-containing medium and swirl gently. Incubate the cells at 37°C in a CO_2 incubator.
7. For transient expression, assay for reporter gene activity 24 to 48 hr after transfection.
8. For stable expression of the transfected plasmid sequence, remove transfection medium 24 hours after transfection and trypsinize the cells. Transfer the cells to a fresh plate with growth medium containing no selective agent. The following day, replace the medium with new medium containing the selective agent. Continue incubating for 1 to 2 weeks to allow growth of the cells expressing the transgene.

Note: COSFectin lipid reagent also gives high transfection efficiency in the absence of serum. If these conditions are required, at the moment of transfection, replace the serum-containing medium in which cells are growing with serum-free medium. Then proceed to prepare complexes as indicated in steps 3 to 6, using the recommended conditions from Table 1.

Protocol for Rapid Transfection of COS Cells
(24-Well Plates)

1. One or two days before transfection, divide the culture so they will be actively growing (80 to 90% confluent) at the time of transfection.
2. In 25 µl of serum-free medium, prepare nucleic acid to have a final concentration of 1.0 to 2.0 µg per well. (for different sized culture vessels, see recommended amounts in Table 1)
3. In a polystyrene container, prepare the COSFectin lipid reagent in 25 µl of serum-free medium.
   (for different sized culture vessels, see recommended volumes in Table 1)
4. Mix the nucleic acid-COSFectin solutions by tapping or pipetting. Incubate 20 minutes at room temperature, to allow complexes to form.
5. Add 50 µl of the nucleic acid/COSFectin mix into the 24-well plate.
6. Within 5 min of adding the complexes to the 24-well plates, add 1.0 to 1.8 x 10^5 cells/well in growth medium. Note: this number should be optimized for the particular COS cell line being used. For the Rapid Transfection protocol, we recommend using 2–2.5 times the number of cells used for a standard transfection.
7. Incubate the cells at 37°C in a CO_2 incubator for 24 hrs prior to assaying for activity.

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