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GenCarrier™ - Cell Transfection Reagents

GenCarrier-1 (Cat. No.: 31-00110)
GenCarrier-2 (Cat. No.: 31-00120)

Store at 4° C or above, DO NOT FREEZE.

I. Description

GenCarrier-1™ has been used successfully to transfect DNA or RNA into CHO-K1, COS, LNCaP, NIH3T3, SF-9, S2 (Schneider -Drosophila), 293, T-24, C2C12, primary human keratinocytes, primary aortic smooth muscle, primary rabbit myoblasts, human bone marrow endothelial cells (HBMEC), and other cell lines. GenCarrier-1™ has been shown to be most optimal for the cell lines tested at a working concentration of 2-6 µg per µg of DNA in 35mm dishes. The reagent works in 10% serum medium.

GenCarrier-2™ - has been used successfully to transfect DNA into COS, HepG2, PC12, NIH3T3, Jurkat, primary rat and hamster hepatocytes, primary rat cardiomyocytes, primary male rat germ cells, primary chick retinal neurons, primary rat fibroblasts, primary human keratinocytes, and tracheobroncial cells. GenCarrier-2™ has been shown to be most optimal for the cell lines tested at a working concentration of 1-4 µg per µg of DNA in 35mm dishes.

II. Working Concentration

GenCarrier-1™ and GenCarrier-2™ are liposomal formulations of a polycationic lipid and a neutral, non-transfecting lipid compound. Enough GenCarrier-1™ or GenCarrier-2™ is provided for approximately 250 transfections in 35mm plates. GenCarrier-1™ and GenCarrier-2™ are provided as a sterile suspension in water at a concentration of 1 mg/ml. The optimal conditions for achieving the highest transfection efficiency involves several parameters including cell confluence, the composition of the DNA/liposomal complex and the duration of transfection.

III. Determining optimal conditions

A. Cell Confluence

Cell growth should cover 50-70% of the total plating area before performing a transfection. In general, it is easier to control the level of growth by using larger well plates.

B. DNA/Liposomal Complex

The optimal DNA to liposome ratio used can be determined by beginning with a 1 µg amount of plasmid DNA per 35 mm well. Vary the amount of GenCarrier-1™ or GenCarrier-2™ over a range of 1-6 µl, in 0.5 or 1.0 µl increments, per 35 mm well.

Once the optimal DNA to liposome ratio for a given cell type is identified, the appropriate amount of complex used per well should be determined. For example, if the optimal DNA/liposome ratio in the initial experiment was 1 µg/1 µl, then amounts ranging from 0.5 µg/0.5 µl, 1.5 µg/1.5 µl and 2.0 µg/2.0 µl should be tested to determine which amount provides the highest level of transfection efficiency.

C. Duration of Transfection

An additional factor to consider when optimizing transfection is the duration that cells are exposed to the DNA/liposome complex. As a general rule, the longer the exposure time, the higher the efficiency rate. However, since transfection is usually performed with low serum-containing or serum-free medium, prolonged exposure to these reagents can result in cell detachment or death. Therefore, it is recommended that an initial transfection time of 5 to 18 hours be used. Longer exposure times may be possible depending on the cell line.

IV. Transfection Protocol- Adherent Cells

A. Cell Plating

The following protocol applies to transfections performed in 35 mm well plates. Adjust volumes proportionately for wells or dishes of different sizes.

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B. Formation of the GenCarrier-1™ or GenCarrier-2™/DNA Complex

1. Isolate plasmid DNA using standard kits available from Epoch Biolabs or a similar manufacturer. Reprecipating the DNA using sodium acetate/ethanol has been shown to increase transfection efficiency. If reprecipitation is performed, it is imperative to wash the DNA pellet repeatedly using ice-cold 70% ethanol to remove salts which could adversely impact transfection efficiency. Once the pellet has dried sufficiently, dissolve it in sterile, deionized water. Adjust the final concentration to 1.0 µg/µl using an absorbance at 260 nm. Store the DNA at - 20° C.

2. Dilute the purified plasmid DNA in sterile serum-free or low-protein medium without antibiotics using 1.5 ml centrifuge tubes. The final concentration of DNA should be 1.0 µg/100 µl.

3. Dilute 0.5, 1.0, 2.0, 4.0 or 6.0 µl amounts of GenCarrier-1™ or GenCarrier-2™ with a 100 µl of low-protein or serum-free medium without antibiotic in siliconized 1.5 ml microcentrifuge tubes.

Note: Unsiliconized tubes can be used, but better results are usually achieved with siliconized tubes. The DNA/liposome complex containing GenCarrier-2™ must be made in serum-free medium. In contrast, the DNA/liposome complex containing GenCarrier-1™ can be formed in the presence of fetal bovine serum that is reduced 20-50% from levels normally used to culture cells.

The use of antibiotics with GenCarrier-1™ or GenCarrier-2™ will adversely impact cell viability and transfection efficiency.

4. Add the plasmid DNA solution directly to the dilute GenCarrier-1™ or GenCarrier-2™ using a pipet. Mix the two solutions by tapping the tubes with your finger or by repeatedly pipeting the liquid. The DNA/liposome complex should form within seconds; however, it is advised to incubate the mixture for at least 15 and as long as 45 minutes at room temperature.

C. Transfection

1. Wash the cell layer twice with 1 to 2 ml amounts of low-protein or serum-free and antibiotic-free medium. *Note: **Do not use PBS** since the residual phosphate may compete with DNA and bind GenCarrier-1™ or GenCarrier-2™ thereby reducing the transfection efficiency.*

2. Add a 0.8 ml amount of low-protein or serum-free and antibiotics-free medium to each well.

3. Add the 200 µl amount of the DNA/liposome complex to each well in a dropwise fashion using a Pipetman® or similar device. Attempt to cover the entire cell layer with the complex. Once the volume has been added, gently mix the solution by swirling the plate to ensure the entire layer is immersed in solution.

4. Incubate the cells for 5 to 18 hours under conditions normally used to culture the cells. Based on several studies, an incubation period of 5-7 hours has been shown to result in the highest level of transfection efficiency with certain cell types; however, other cell lines may require longer incubation periods.

5. Following incubation, add a 1 ml amount of medium containing 2 times the normal serum and antibiotics concentration (2x medium). At this point, serum can be added to a serum-free or low protein medium. *Notes: Addition of 2x medium has been shown to result in a higher level of transfection. Furthermore, the 2x medium restores cells to their normal growth conditions*.

6. Incubate the cells an additional 18-24 under conditions normally used to culture the cells.

7. Aspirate the medium and replace with fresh 1x medium containing the additives normally used to culture the cells.

8. Assay the cells using the appropriate protocol 24-72 hours after the addition of fresh medium in Step 7.

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V. Transfection in the presence of serum

A. GenCarrier-1™ ONLY

1. Make the DNA:liposome complex in serum-free medium as above in 1 ml total volume at room temperature for 15 min.

2. Add 1ml of medium containing 20% FBS to the above complex (final FBS concentration is 10%).

3. Add 2 ml/each of the solution of step 2 to the freshly aspirated 35mm dishes containing cells at 80% confluence.

4. Incubate cells overnight.

5. Aspirate the medium and replace it with 2 ml fresh medium containing 10%FBS. Incubate for another 24hrs.

6. Assay the cells using the appropriate protocol 24-72 hours later.

VI. Transfection Protocol- Suspension Cells

A. Cell Preparation

1. Transfer a suspension cell culture to 50 ml conical tubes and centrifuge at 400 X *g* for 10 minutes.

2. Wash twice the cell pellet with low-protein or serum-free and antibiotics-free medium by aspirating the supernatant and gently re-suspending the cell pellet in 10-20 ml of medium.

3. Re-suspend the cell pellet to a final concentration of 6 X 10^6 cells/ml in low-protein or serum-free and antibiotics-free medium.

4. Transfer 0.8 ml amount of cell suspension to a 35mm well and incubate under conditions normally used to culture the cells. Adjust volumes proportionately for different sized wells or dishes.

B. Formation of the GenCarrier-1™ or GenCarrier-2™/DNA Complex

**The DNA/liposome complex should be prepared at room temperature.*

1. Isolate plasmid DNA using standard kits available from Epoch Life Science or a similar manufacturer. Re-precipitating the DNA using sodium acetate/ethanol has been shown to increase transfection efficiency. If re-precipitation is performed, it is imperative to wash the DNA pellet repeatedly using ice-cold 70% ethanol to remove salts which could adversely impact transfection efficiency. Once the pellet has dried sufficiently, dissolve it in sterile, deionized water. Adjust the final concentration to 1.0 µg/µl using absorbance at 260 nm. Store the DNA at - 20° C.

2. Dilute the purified plasmid DNA using sterile low-protein or serum-free and antibiotics-free medium in 1.5 ml centrifuge tubes. The final concentration of DNA should be 1.0 µg/100 µl.

3. Dilute 0.5, 1.0, 2.0, 4.0 or 6.0 µl amounts of GenCarrier-1™ or GenCarrier-2™ with a 100 µl of low-protein or serum-free and antibiotics-free in siliconized 1.5 ml microcentrifuge tubes.

Note: Unsiliconized tubes can be used, but better results are usually achieved with siliconized tubes. The DNA/liposome complex containing GenCarrier-2™ must be made in serum-free medium. In contrast, the DNA/liposome complex containing GenCarrier-1™ can be formed in the presence of fetal bovine serum that is reduced 20-50% from levels normally used to culture cells.

The use of antibiotics with GenCarrier-1™ or GenCarrier-2™ will adversely impact cell viability and transfection efficiency.

4. Add the plasmid DNA solution directly to the dilute GenCarrier-1™ or GenCarrier-2™ using a pipet. Mix the two solutions by tapping the tubes with your finger or by repeatedly pipeting the liquid. The DNA/liposome complex

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should form within seconds; however, it is advised to incubate the mixture for at least 15 and as long as 45 minutes at room temperature.

C. Transfection

1. Following the 15-45 minute incubation period, add the 200 µl of the DNA/liposome to a well in a dropwise fashion using a Pipetman® or similar device. Once the complex has been added, gently mix the solution by swirling the plate to ensure the DNA/liposome complex is in solution.

2. Incubate the cells for 5 to 18 hours under conditions normally used to culture the cells. Based on several studies, an incubation period of 5 to 7 hours has been shown to result in the highest level of transfection with certain cell types; however, other cell lines may require longer incubation periods.

3. Following incubation, add 4 ml of medium containing 12.5% FBS to each well and continue the incubation for an additional 72 hours. Assay the cells using the appropriate protocol for gene expression.

Stability and Storage:
GeneCarrier™ Is a Research Use Only product and is not intended for *In Vitro* Diagnostic Use. The product should be stored 4°C or above and protected from light. Normal precautions should be taken when working with this or any other research product. A Material Safety Data Sheet is available upon request.

Storage Temperature: Store at 4°C or above.
DO NOT FREEZE

Related Products:
RNAiCarrier™: Cat. No. 31-00130, 1ml