

DMRIE-C Reagent

Cat. No.: 10459-014
Conc.: 2 mg/ml

Size: 1 ml
Store at 4°C.
DO NOT FREEZE. MIX WELL BEFORE USE.

Description:

DMRIE-C Reagent is a 1:1 (M/M) liposome formulation of the cationic lipid DMRIE (1,2-dimyristyloxypropyl-3-dimethyl-hydroxy ethyl ammonium bromide) (1) and cholesterol in membrane-filtered water. It is suitable for the transfection of DNA and RNA into tissue culture cells. One milliliter is sufficient for 80 to 500 transfections on 35-mm tissue culture dishes or 25 to 150 transfections on 60-mm dishes. DMRIE-C Reagent interacts spontaneously with DNA to form a lipid-DNA complex. DMRIE-C Reagent was found to be superior for the transfection of DNA in Jurkat (human T-cell leukemia), K-562 and KG-1 (human myelogenous leukemia), and MOLT-4 (human lymphoblastic leukemia) cell lines and for the transfection of RNA in adherent cell lines (2, 3). DMRIE-C Reagent is also an effective compound for the transfection of other cell lines such as CHO-K1, COS-7, NIH-3T3, and BHK-21 (3). The cationic lipid DMRIE has been used for *in vivo* delivery of DNA (4).

Transfection Optimization:

The most important aspect of successful transfection is the careful optimization of transfection conditions for each cell type. We recommend that the optimal amount of DMRIE-C Reagent, DNA concentration, and incubation time of DMRIE-C Reagent-DNA complexes with cells be determined for each cell line. Cell density must also be optimized and kept consistent to obtain reproducible results. The amount of DMRIE-C Reagent can be optimized first, using a constant amount of DNA. We recommend starting with 1-4 µg DNA for 35-mm culture dishes and a 5 h incubation time. With these two parameters held constant, vary the amount of DMRIE-C Reagent to determine the optimal concentration of lipid (usually 2-12 µl). The amount of DNA, as well as time of incubation of cells with the DMRIE-C Reagent-DNA complexes (2-24 hours), can also be optimized. The concentrations of DNA and DMRIE-C Reagent and transfection times in the following transient protocols were determined with the plasmids pCMV•SPORT-βgal, or pCMV-CAT in CHO-K1, BHK-21, COS-7, Jurkat, and K562 cells. RNA transfections were optimized using *in vitro* transcribed SFV-lacZ RNA (5). These conditions are guidelines only.

Quality Control:

1. DMRIE-C Reagent is tested for the absence of bacterial and fungal contamination using blood agar plates and fluid thioglycolate medium.
2. A functional assay using DMRIE-C Reagent involves the transfection of Jurkat cells with pCMV.SPORT.LUC DNA. The cells are assayed for luciferase activity using a modification of the procedure of Wood (6).
3. DMRIE-C Reagent contains no detectable RNase activity using Globin mRNA as a substrate.

Protocols:

Transient Transfection of Adherent Cells:

1. In a six-well or 35-mm tissue culture plate, seed $\sim 2 \times 10^5$ cells in 2 ml of the appropriate growth medium supplemented with serum.
2. Incubate the cells at 37°C in a CO₂ incubator until the cells are 60-80% confluent. This will usually take 18-24 h, but the time will vary among cell types. (**Note:** For the cells we have evaluated, 60-80% confluency is optimal, however for other cell lines, optimal cell density may vary. Since transfection efficiency may be sensitive to culture confluency, it is important to maintain a standard seeding protocol from experiment to experiment).
3. Prepare the following solutions in 12 x 75-mm sterile tubes:
Solution A: For each transfection, dilute 1-2 µg of DNA into 500 µl serum-free medium. Opti-MEM® I Reduced Serum Medium (Cat. No. 31985) gives optimal results, however, other serum-free media can also be used.
Solution B: For each transfection, dilute 2-12 µl of DMRIE-C Reagent (Mix well; see note) into 500 µl serum-free medium.
Note: DMRIE-C Reagent is a lipid suspension that may settle with time. Mix thoroughly by inverting the tube 5-10 times before removing a sample for transfection to ensure that a homogenous sample is taken.
Note: Some serum-free media formulations can inhibit cationic lipid-mediated transfection. Test media for compatibility with transfection reagent before use.
Note: When transfecting with higher amounts of DNA or DMRIE-C Reagent, increase the volume of Opti-MEM® I Reduced Serum Medium proportionally. For optimal transfection, do not exceed 20 µg/ml DNA or 200 µg/ml DMRIE-C Reagent. When transfecting different sized tissue culture plates, change the amounts of DNA, DMRIE-C Reagent and medium in proportion to the difference in surface area.
4. Combine the two solutions, mix gently, and incubate at room temperature for 15-45 min. The solution may appear cloudy, however this will not interfere with the transfection.
5. Wash the cells once with 2 ml of serum-free medium (optional).
6. Remove medium and overlay the lipid-DNA complex solution onto cells.
7. Incubate the cells for 4-24 h at 37°C in a CO₂ incubator.
8. Add 1 ml of growth medium containing twice the normal concentration of serum without removing the DNA-containing medium and incubate cells at 37°C in a CO₂ incubator for a total of 24-72 h. (Alternatively, or if toxicity is a problem, replace the DNA-containing medium with 2 ml of normal growth medium containing serum normal growth medium.)
9. Assay the cells for the appropriate activity at 24-72 h post transfection.

Transient Transfection of Suspension Cells:

1. To each well of a six-well plate add 0.5 ml Opti-MEM® I Reduced Serum Medium.

Doc.Rev.: 08/23/01

This product is distributed for laboratory research use only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Invitrogen TECH-LINESM 800 955 6288

2. Add 0, 2, 4, 6, 8, or 12 μ l of DMRIE-C Reagent (Mix well; see note) to each well, and mix gently by swirling plate.
Note: DMRIE-C Reagent is a lipid suspension that may settle with time. Mix thoroughly by inverting the tube 5-10 times before removing a sample for transfection to ensure that a homogenous sample is taken.
3. Add 0.5 ml Opti-MEM[®] I Reduced Serum Medium containing 4 μ g DNA to each well. Mix by swirling plate.
4. Incubate the plate at room temperature for 15-45 min to allow formation of the lipid-DNA complexes.
5. Add 2×10^6 cells in 0.2 ml serum-free medium to each well. Mix gently.
6. Incubate 4-5 h at 37°C in a CO₂ incubator.
7. To each well add 2 ml growth medium containing 15% FBS. (For Jurkat and MOLT-4 cells, addition of PHA and PMA at final concentrations of 1 μ g/ml and 50 ng/ml, respectively, enhances promoter activity and gene expression. For K562 and KG-1 cells, PMA alone enhances promoter activity.)
8. Assay the cells at 24 or 48 h post-transfection for the appropriate activity.
Note: When transfecting different sized tissue culture plates, change the amounts of DNA, DMRIE-C Reagent and medium in proportion to the difference in surface area.

Transfection of RNA:

1. In a six-well or 35-mm tissue culture plate, seed $\sim 2-3 \times 10^5$ cells in 2 ml of the appropriate growth medium supplemented with serum.
2. Incubate the cells at 37°C in a CO₂ incubator until the cells are $\sim 80\%$ confluent. This will usually take 18-24 h, but the time will vary among cell types. (**Note:** For the cells we have evaluated, 80% confluency is optimal, however for other cell lines, optimal cell density may vary. Since transfection efficiency may be sensitive to culture confluency, it is important to maintain a standard seeding protocol from experiment to experiment).
3. Wash the cells in each well with 2 ml of Opti-MEM[®] I Reduced Serum Medium (room temp.).
4. Preparation of RNA-Lipid Complexes:
 - a. Add 1.0 ml Opti-MEM[®] I (room temp.) to each of six 12 x 75 mm polystyrene tubes.
 - b. Add 0, 2, 4, 6, 8, or 12 μ l DMRIE-C Reagent (Mix well; see note) to each of the tubes containing Opti-MEM[®] I Medium, and mix or vortex briefly.
Note: DMRIE-C Reagent is a lipid suspension that may settle with time. Mix thoroughly by inverting the tube 5-10 times before removing a sample for transfection to ensure that a homogenous sample is taken.
 - c. Add 2.5 - 5.0 μ g RNA to each tube in step 4b and vortex briefly.
5. Immediately add Lipid-RNA complexes (from step 4c) to washed cells.
6. Incubate 4 h at 37°C, then replace transfection medium with complete growth medium.
Note: The time of exposure of cells to Lipid-RNA complexes, as well as the amount of RNA added to cells should be adjusted for each cell type.
7. Allow the cells to express the RNA for 16-24 h and analyze them for expression of the transfected RNA.
Note: mRNA that is capped and polyadenylated is translated more efficiently and is more stable within the cell.

Stable Transfection of Adherent Cells:

1. Seed $1-2 \times 10^5$ cells/60-mm tissue culture plate in 4 ml of the appropriate growth medium supplemented with serum.
2. Incubate the cells at 37°C in a CO₂ incubator for 18-24 h. Cells should be 30-50% confluent.
3. Prepare the following solutions in 12 x 75-mm sterile tubes:
Solution A: For each transfection dilute 2 μ g DNA into 1 ml Opti-MEM[®] I Medium.
Solution B: For each transfection dilute 2-12 μ l of DMRIE-C Reagent (Mix well; see note) into 1 ml Opti-MEM[®] I Medium.
Note: DMRIE-C Reagent is a lipid suspension that may settle with time. Mix thoroughly by inverting the tube 5-10 times before removing a sample for transfection to ensure that a homogenous sample is taken.
4. Combine the two solutions, mix gently, and incubate at room temperature for 15-45 min to allow formation of the lipid-DNA complexes.
5. Wash the cells once with 2 ml of serum-free growth medium without antibacterial agents (optional).
6. Remove medium and overlay the lipid-DNA complex solution onto the cells.
7. Incubate the cells for 5-24 h at 37°C in a CO₂ incubator.
8. Replace the DNA containing medium with 4 ml of growth medium supplemented with the normal percentage of serum and incubate the cells at 37°C in a CO₂ incubator for another 48 h.
9. Subculture the cells at desired ratios, at least 1:5, into selection medium.

Transfection in the Presence of Serum:

Successful transfection with DMRIE-C Reagent may be achieved in the presence of serum providing that lipid-DNA complexes are formed in serum free medium (**Note:** See steps 1-4 in above procedures). Antibacterial agents should not be added to cells in the medium together with the DNA-lipid complexes. We recommend Dulbecco's Modified Eagle Medium (Cat. No. 11960), Opti-MEM[®] I Reduced Serum Medium or other serum-free growth medium supplemented with 5-10% fetal bovine serum. In the presence of 5-10% serum, there should be little effect on transfection efficiency. A variation in reporter gene activity ($\pm 15\%$) may be seen depending on transfection conditions.

Note:

Some serum-free media formulations can inhibit cationic lipid-mediated transfection. Test any new serum-free formulation for compatibility with the transfection reagent prior to use. Media formulations that have been found to inhibit transfections are: CD 293 Medium, 293 SFM II, and VP-SFM.

References:

1. Felgner, J.H., Kumar, R., Sridhar, C.N., Wheeler, C.J., Tsai, Y.J., Border, R., Ramsey, P., Martin, M., and Felgner, P.L. (1994) *J. Biol. Chem.* 269, 2550.
2. Ciccarone, V., Anderson, D., Lan, J., and Jessee, J. (1995) *Focus*[®] 17, 84.
3. Macdonald, A. S., Schifferli, K., Anderson, D., Jessee, J., and Ciccarone, V. (1996) *Focus*[®] 18, 6.
4. Logan, J.J. et al. (1995) *Gene Therapy* 2, 38.
5. Ciccarone, V., Anderson, D., and Jessee, J. (1994) *Focus*[®] 16, 94.
6. Wood, K.V. (1991) in *Bioluminescence and Chemiluminescence: Current Status*, (P. Stanley and L. Kricka, eds.) John Wiley and Sons, Chichester, p. 543.

For references and protocols pertaining to transfection of your cell type, please see our web site at <http://www.invitrogen.com/transfection/celltypes/>

This product is the subject of U.S. Patents 5,459,127 and 5,264,618.

Cat. No.: 10459-014