MDCK Cell Avalanche® Transfection Reagent

Cat. No. EZT-MDCK-1 **Size: 0.5 ml** **Store at 4°C**
1.5 ml

Cell Line Information:

Designations: MDCK
Cellular Products: keratin
Isolation date: September, 1958
Applications: transfection host
Virus Susceptibility: Human Coxsackievirus B, Human Coxsackievirus B, Human Coxsackievirus B, Human poliovirus.
Age: adult
Gender: female
Comments: The MDCK cell line was derived from a kidney of an apparently normal adult female cocker spaniel, September, 1958, by S.H. Madin and N.B. Darby. The cells are positive for keratin by immunoperoxidase staining. MDCK cells have been used to study processing of beta amyloid precursor protein and sorting of its proteolytic products.

The Transfection Reagent:

MDCK Cell Avalanche® Transfection Reagent (Hereafter “The Reagent”) is a new, proprietary solution specifically designed for transfection on MDCK Cells. The proprietary formulation of lipids and polymers ensures the highest possible transfection efficiencies and viabilities for MDCK Cells.

Features:

- Unique formulation-maximize transfection performance in MDCK Cells.
- Lowest Cellular Toxicity-maintain cell density and reduce experimental biases
- Deliver single or multiple plasmids
- Easy-to-use protocol: Just mix nucleic acids with the reagent to form complexes and simply add the mixture to cells
- Suitable for Reverse Transfection
- Compatible with transfection in any plate formats
- Reproducible: due to highly controlled chemical synthesis of each of the ingredients, the reagent forms uniformly sized complex particles with nucleic acids. With optimized protocol, our reagent will ensure the reproducible highest transfection results.
- Economical: High efficiency means less amount of nucleic acid & reagent is needed
- Developed and manufactured by EZ Biosystems

BEFORE YOU START:

Important Tips for Optimal Transfection

1. Prepare high-quality plasmid DNA at 0.5–5 µg/µl in deionized water or TE buffer. A GFP (green fluorescent protein) plasmid can be used to determine transfection efficiency.
2. Use Opti-MEM® I Reduced Serum Medium (Life Technologies) or regular DMEM without serum to make The Reagent and nucleic acid mix. Do not use $NaCl_2$ solution or PBS.
3. Maintain the same seeding conditions between experiments. Use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection.
4. The Reagent is extremely gentle to cells. However, transfection process will impose stress on cells, no matter what type of transfection methods you use. The trick is to get the balance between transfection efficiency and cell viability. Increasing the number of cells plated per well or decreasing The Reagent amount will minimize the effect of transfection on cell growth and viability. With careful adjustment, as described in page 3 and 4, this can be achieved while keeping the highest transfection efficiency.
5. Don't use antibiotics in the culture medium during the first 24 hours of transfection.

Protocols

1 DNA Transfection

1.1 Cell Seeding

For optimal DNA transfection conditions, we recommend using cells which are 70% to 90% confluent at the time of transfection. Typically, for experiments in 6-well plates, 150,000-

250,000 cells are seeded per well in 2 ml of cell growth medium **without antibiotics** 24 h prior to transfection. For other culture formats, refer to Table 1.

Table 1. Recommended number of cells to seed the day before transfection

Culture vessel	Number of cells to seed	Surface area per well (cm^2)	Volume of medium per well to seed the cells (ml)
96-well	7,500-10,000	0.3	0.1
24-well	50,000-80,000	1.9	0.5
12-well	80,000-150,000	3.8	1
6-well/35 mm	150,000-250,000	9.4	2
60 mm/flask 25 cm^2	250,000-800,000	25-28	5
100 mm/flask 75 cm^2	$1x10^6$-$2x10^6$	75-78.5	10
150 mm/flask 175 cm^2	$2x10^6$-$5x10^6$	153-175	25

1.2 DNA Transfection on MDCK cells

Due to cell culture variations and passage number differences, MDCK cells from different sources may have different sensitivity to The Reagent. If this is the first time that you are using The Reagent on your MDCK cells, transfect the cells according to Table 2a for pre-optimization (**The pre-optimization procedures are extremely important for successful transfection. The amount of The Reagent needed for maximum transfection on MDCK cells from different sources may differ dramatically**).

Table 2a. Transfection guidelines according to the cell culture vessel per well

Component	**96-well**	**24-well**	**6-well**
Opti-MEM Medium (µl)	250	250	1200
DNA (µg)	2.5	2.5	12
Diluted DNA (µl)	4 x 50 µl	4 x 50 µl	4 x 250 µl
The Reagent (µl)	*0.2, *0.4, *0.7, 1.0	*0.2, *0.4, *0.7, 1.0	1.0, 2.0, 3.5, 5.0
Incubate for 15 minutes at room temperature			
DNA-reagent complex/well (µl)	**10**	**50**	**250**
Immediately centrifuge the plate at 300 g for 5 min			
Gently put in incubator, and incubate cells for 24 hours or more at 37°C before analysis			

*Dilute The Reagent 1:5 with H_2O prior application (4 µl reagent + 16 µl H_2O), and then use 5 times of volume for accurate pipetting.

Table 2b shows the amounts of DNA and The Reagent per well used in each of the above transfection reactions.

Table 2b. Amount of DNA and The Reagent per well

Amount	96-well	24-well	6-well
DNA/well (ng)	100	500	2500
The Reagent (µl)	0.04-0.2	0.2-1.0	1.0-5.0

As an example, the following steps are given for optimization on 6-well plate. For other culture formats, please refer to Table 2 and Table 3.

1. Transfer 12.0 µg DNA into 1200 µl Opti-MEM® Reduced-Serum Medium (Cat# 31985-070, Life Technologies) or regular high glucose DMEM without serum. Mix by vortexing. Aliquot 4 x 250 µl of the above DNA solution into 4 x 1.5 ml Eppendorf tubes.
2. Briefly vortex The Reagent, and add 1.0, 2.0, 3.5, and 5.0 µl into the above diluted DNA respectively. Immediately vortex for 5 s after each addition.
3. Incubate for 15 min at RT.
4. Add the 250 µl transfection mixture drop-wise into each well (Note: for the 96-well format, the amount of transfection mixture added per well is only part of the total volume as indicated in Table 2).
5. Gently rock the plates back and forth and from side to side, and **immediately centrifuge the plate at 300 g for 5 min**.
6. Gently put in incubator, and incubate at 37 °C CO_2. It is not necessary to remove complexes or to change/add medium after transfection.
7. Analyze after incubating for 24 h or longer.

After you have completed the optimization steps, choose the amount of The Reagent that gave you the optimal balance of potency & low cytotoxicity from the above procedure for all of your future experiments on your MDCK cells.

1.3 Scale Up or Down Transfections

Use Table 3 to scale the volumes for your transfection experiment.

Table 3. Scaling Up or Down Transfection Instruction

Culture Vessel	[1]Multiplication factor	Vol. Complex-Opti-MEM per well (µl)	DNA (µg)	[2]The Reagent (µl)
96-well	0.17	10	0.1	0.04-0.2
48-well	0.50	25	0.25	0.1-0.5
24-well	**1.00**	**50**	**0.5**	**0.2-1.0**
12-well	2.00	100	1.0	0.4-2.0
6-well	5.00	200	2.5	1.0-5.0
60-mm	11.05	500	5.5	2.3-11.5
10-cm	28.95	1000	14	5.8-29
T75	39.47	1500	20	7.9-39

[1]After determining the optimum reagent amount, use the multiplication factor to determine the reagent amount needed for your new plate format.

[2]Optimum amount needed is determined from the protocol in the previous two pages.

1.4 DNA Transfection on cells other than MDCK cells

MDCK Cell Avalanche® Transfection Reagent can also be used on the following cells with high transfection efficiencies.

786-O Cell, Caki-1 Cell, Vero Cell, 293 Cell, 293T/17 Cell

The above protocol usually provides satisfactory transfection efficiency with invisible cytotoxicity on the above cells. However, optimization may be needed for certain type of cells. Optimizations may include: cell density; the amount of DNA and The Reagent; DNA/The Reagent ratio, or incubation time for the mixture of The Reagent/DNA etc.

If you want to have a broad spectrum transfection reagent that provides excellent transfection efficiency on most of your cell types for day-to-day experiments with minimum optimization, Avalanche-Omni-Transfection Reagent (EZT-OMNI-1) is the one that you need.

If you want to achieve the best transfection result for a specific type of cells, we recommend using the respective cell type/cell line specific Avalanche® transfection reagents. Those reagents have been optimized on both recipes and protocols, and have been proved to have the best transfection results for the respective cell lines or primary cells. You can easily find the respective Avalanche® Transfection Reagents specific for your cells by using the filters of our product list in EZ Biosystems website: www.ezbiosystems.com.

2 siRNA Transfection

Though MDCK Cell Avalanche® Transfection Reagent formulation was optimized only for DNA transfection, it can also be used for siRNA transfection. The following are recommended protocol. Protocol optimization may be needed.

2.1 Cell Seeding

For optimal siRNA transfection conditions, we recommend using cells which are 50% confluent at the time of transfection. Typically, for experiments in 6-well plates, 100 000 to 150 000 cells are seeded per well in 2 ml of growth medium without antibiotics 24 h prior to transfection. For other culture formats, refer to Table 4.

Table 4. Recommended number of cells to seed the day before transfection.

Culture vessel	Number of cells to seed	Surface area per well (cm^2)	Medium per well to seed the cells (ml)
24-well	25,000-40,000	1.9	0.5
12-well	50,000-80,000	3.8	1
6-well/35 mm	100,000-150,000	9.4	2
60 mm/flask 25 cm^2	200,000-500,000	25-28	5
100 mm/flask 75 cm^2	$0.5x10^6$-$1x10^6$	75-78.5	10

2.2 siRNA Transfection

We recommend using 10 to 50 nM siRNA (final concentration). The following conditions are given per well of a 6-well plate. For other culture format, please refer to Table 5.

1. Dilute 22 to 110 pmoles siRNA (final concentration: 10 to 50 nM) into 200 µl of Opti-MEM® Reduced-Serum Medium or regular high glucose DMEM without serum. Mix by vortexing.
2. Briefly vortex The Reagent, and add 1.0-5.0 µl into the diluted siRNA. Immediately vortex for 10 s.
3. Incubate for 15 min at RT.
4. Add the transfection mixture drop-wise into each well.
5. Gently rock the plates back and forth and from side to side, and **immediately centrifuge the plate at 300 g for 5 min**.
6. Gently put in incubator, and incubate at 37 °C CO_2.
7. Analyze after 24 h or later.

Table 5. siRNA transfection guidelines according to the cell culture vessel per well

Culture Vessel	siRNA (pmole) 10 nM	siRNA (pmole) 50 nM	The Reagent (µl)	Opti-MEM or DMEM (µl))	Growth medium (ml)	Final Volume in the well (ml)
24-well	5.5	27.5	*0.2-1.0	50	0.5	0.55
12-well	11	55	*0.4-2.0	100	1	1.1
6-well/ 35 mm	22	110	1.0-5.0	200	2	2.2
60 mm/ flask 25 cm^2	44	220	2.3-11.5	400	4	4.4
100 mm/ flask 75 cm^2	121	605	5.8-29	1100	11	12.1

* Dilute The Reagent 1:5 with H_2O prior application (4 µl reagent + 16 µl H_2O), and then use 5 times of volume for accurate pipetting.