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Datasheet

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ACExFect 2000 Transfection Reagent

Cat# CC1003 – 1 ml / CC1004 – 5 ml

Storage at 4℃ and DO NOT FREEZE!

INTRODUCTION

ACExFect 2000 Transfection Reagent is a novel and efficient transfection reagent based on cationic liposomes, which is suitable for DNA transfection and co-transfection systems for most eukaryotic cells (both adherent and suspended). The ACExFect 2000 has a unique structure and an optimized formulation. The presence of serum and antibiotics will not affect the efficiency of transfection, thereby reducing the effect of serum-deprival on cells. The ACExFect 2000 is also with low cytotoxicity. After transfection, there's no need to remove nucleic acid- ACExFect 2000 complex or replace medium within 24 hr-48 hr. The high transfection efficiency of ACExFect 2000 transfection reagent has been validated in a board range of eukaryotic cell lines.

CONTENTS

No	Component	CC1003	CC1004
AA	ACExFect 2000 Transfection Reagent	1 ml	5 ml

APPLICATION

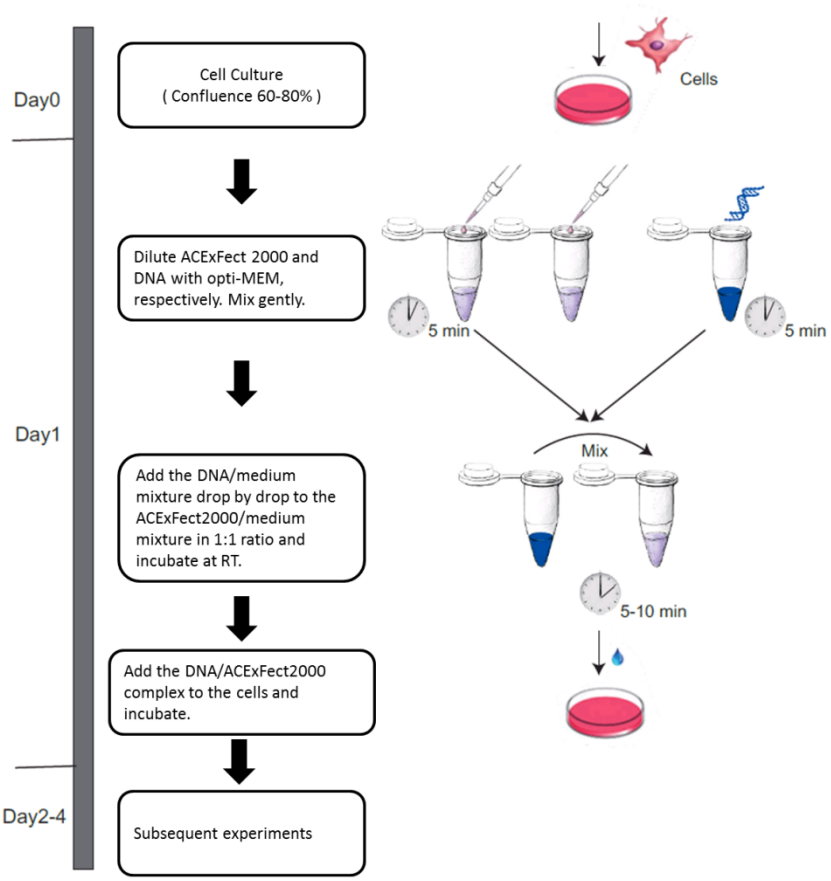
The ACExFect 2000 has been validated in the following cell lines: HEK 293, 293T, A549, B16F10, HCT116, WRL68, MDA-MB-231, Vero, HeLa, Hepa 1-6, Hepa 1cLc7, HepG2, BHK-21, BNLCL.2, C2C12, C6, CHO K1, COS-1, COS-7, Daoy, DU 145, K562, KB, LL/2 (LLC1), LNCaP-FGC, MCF-7, MEL, Neuro 2a, NIH3T3, OVCAR-3, PC-3, and PC-12.

PROTOCOL (Transient Transfection of Adherent/Suspension cells in 24-well plates)

Note :

1. Gently turn the tube upside down to mix thoroughly before use. Vortex if there are insoluble substances in the reagent. If they are still not dissolved, please use after centrifugation at 1000 rpm.
2. Use high quality DNA (with high purity, sterile, and endotoxin-free) for transfection.
3. Avoid the presence of serum during this packaging reaction, as serum interferes with the formation of ACExFect2000/DNA complex.

Overview



1. Cell Culture

1) One day before transfection, plate appropriate number of cells so that cells will be 60% - 80% confluent at the time of transfection.

*Generally, the highest transfection efficiency can be obtained when cell density is 60%-80%. However, the optimal cell density, which varies between different cell lines, can be determined via pre-experiments.

2. Prepare the DNA/ACExFect2000 mixture

1) Add 25 µl of serum-free medium into a 1.5 ml sterile centrifuge tube. Then add ACExFect2000 with an appropriate volume (1 µl-3 µl ACExFect2000/µg DNA, refer to Table 1). Mix gently by pipetting and incubate at room temperature for 5 min.

		96 well	48 well	24 well	12 well	6 well	10 cm dish
Surface Area (cm^2)		0.35	1	1.9	3.8	9.6	59
Complex	**Serum-free medium (µl)**	10	25	50	100	250	2000
	ACExFect2000 (µl)	0.4	1	2	4	10	60
	1 µg/µl DNA (µl)	0.2	0.5	1	2	5	30
Complete Growth Medium(ml)		0.1	0.25	0.5	1.0	2.0	10

2) Add 25 µl of serum-free medium into a 1.5 ml sterile centrifuge tube. Then, add DNA with an appropriate volume (0.5 µg-1 µg DNA/well, refer to Table 1). Mix gently by pipetting and incubate at room temperature for 5 min.

3) Add the DNA/medium mixture drop by drop to the ACExFect2000/medium mixture. Mix gently by pipetting and incubate at room temperature for 5-10 min. Start transfection immediately.

*Ratio of DNA/ACExFect2000: It is recommended to start with an initial ratio of 1:2 or 1:3 (i.e. mix 1 µg of DNA with 2 µl or 3 µl of ExFect2000). Adjust this ratio from 1:1 to 1:3 to obtain the optimized transfection efficiency. The DNA/ACExFect2000 complex should be used within 60 min.

*Do not reverse the mixing order of the ACExFect2000/medium mixture and the DNA/medium mixture.

3. Transfection

1) Add the DNA/ACExFect2000 complex drop by drop to each well containing cell and medium. Mix gently rocking the plate back and forth.

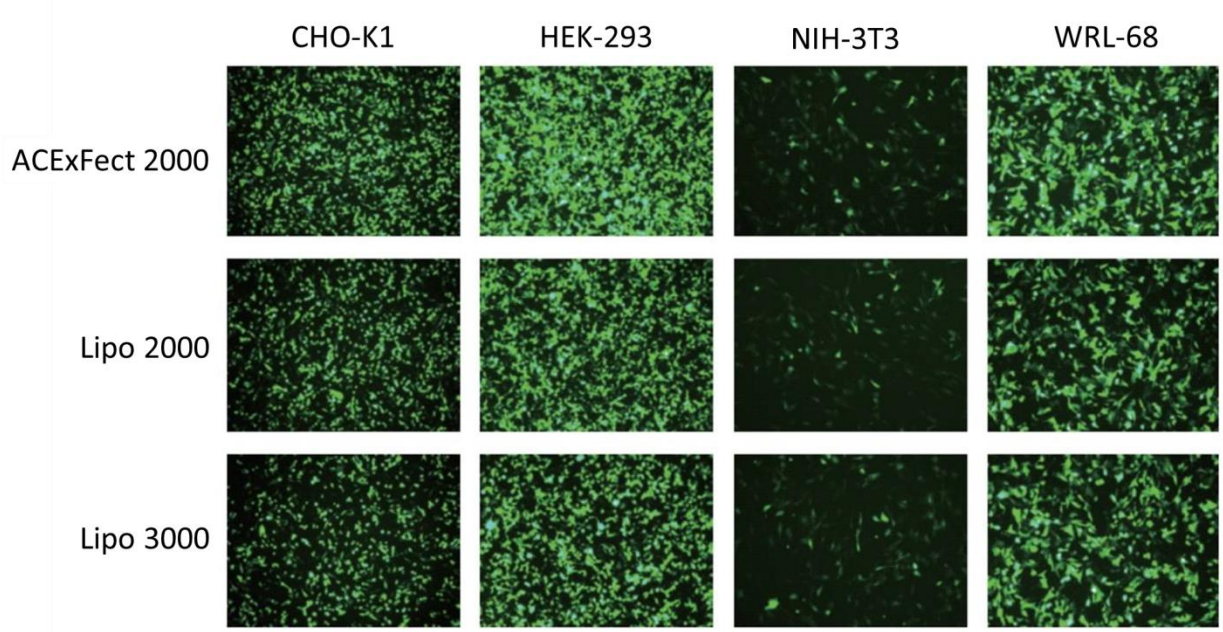
* If necessary, replace the culture medium before transfection.

2) Incubate cells in a CO_2 incubator for 24 to 48 hours.

3) Harvest cells for subsequent experiments.

* Incubation time after transfection: Incubation of 24 to 48 hours is enough for most cell lines. In special cases, the incubation time can be optimized within 12 to 72 hours.

4. Results



TROUBLESHOOTING

Low transfection efficiency	**1. The ratio of DNA/ACExFect2000 or the amount of DNA is not optimized.** Ans. Optimize the ratio of DNA/ACExFect2000 (in a range of 1 µl-3 µl ExFect2000/µg DNA) and the optimal DNA amount (in a range of 0.5 µg-1 µg/well for a 24-well plate) by pre-experimentation. For more DNA amounts, refer to Table 1. Select a ratio with high transfection efficiency and low cytotoxicity for transfection.
	2. The ACExFect2000 and DNA are mixed directly with serum-free medium. Ans. Dilute ACExFect2000 and DNA respectively in serum-free medium, and then incubate together.
	3. The cell density is not optimal. Ans. Generally, the highest transfection efficiency can be obtained when cell density is 60%-80%. However, the optimal cell density, which varies between different cell lines, can be determined via pre-experiments.
	4. Low quality DNA (degrade or containing endotoxin). Ans. DNA used for transfection should be of high purity, sterile, and of no endotoxin.
	5. The DNA/ACExFect2000 complex packaging system contains serum. Ans. Serum interferes with the formation of the DNA/ACExFect2000 complex.
	6. Transfection inhibitory factors exist in the transfection system. Ans. Transfection will not proceed normally with polyanionic polymers (i.e. dextran sulfate, heparin) in the transfection system. It is recommended to use media without these components.
	7. Poor cell status Ans. Select moderately passaged cell lines and maintains the same passage times in different experiments for high efficiency and low cytotoxicity.
Poor cell status after transfection	**1. Long incubation time after transfection with DNA/ACExFect2000 complex.** Ans. In most cases, there's no need to replace the medium in 24 hours after transfection. However, prolonged incubation may lead to poor cell status. It is recommended to arrange the follow-up experiment time according to demands.
	2. Cell density is not optimal Ans. Generally, the highest transfection efficiency can be obtained when cell density is 60%-80%. Low cell density leads to slow cell growth and higher sensitivity on foreign stimuli. While high cell density leads to contact inhibition and accelerated apoptosis.
	3. The ratio of DNA/ACExFect2000 or the amount of DNA is not optimized. Ans. Excessive transfection reagent results in higher cytotoxicity. Try to reduce the use of transfection reagents or reduce the amount of DNA (refer to Table 1).
	4. Unevenly distribution of DNA/ACExFect2000 complex in culture medium. Ans. Excessive regional distribution of DNA/ACExFect2000 complex is one of the most common causes of high cytotoxicity. After adding the complex to culture medium, mix gently rocking the plate back and forth for several times.

	5. Poor cell status Ans. Select moderately passaged cell lines and maintains the same passage times in different experiments for high efficiency and low cytotoxicity.

PRODUCT USE LIMITATION

These products are intended for research use only.