RW 05/11

Protocol

Theme: Double transfection of Keratinocytes with Lipofectamine™ 2000 Invitrogen

Equimpent: Lipofectamin™ 2000 Invitrogen, DNA/plasmid of interest, medium (full medium, reduced medium, minimal medium), 24-well plate

Procedure: for 24-well format, all amounts and volumes are given on a per well basis, composition of medium is given on the next page

1. 1 day before transfection, plate 1,2 x 10^5 cells/well in 500 µl **reduced medium** and incubate at 32°C and 5 % CO_2.

2. Next day discard the old medium, wash twice with **minimal medium** and add 500 µl **minimal medium** to each well.

3. **For each transfection sample**, prepare complexes as follows:

   a. Dilute **1,6 µg precipitated DNA** in 50 µl **minimal medium** and mix gently.

   b. Mix Lipofectamine™ 2000 gently before use, then dilute **2 µl Lipofectamin** in 50 µl of **minimal medium**. Incubate for 5 minutes at room temperature.

   c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine™ 2000 (total volume = 100 µl). Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy).

   Note: Complexes are stable for 6 hours at room temperature.

4. Add the 100 µl of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.

5. Incubate cells at 32°C and 5 % CO_2 for 6 hours.

6. Change medium after 6 hours to **reduced medium**.

7. Incubate cells at 32°C and 5 % CO_2 over night.

8. Next day retransfect the cells as described above (**Step 2-5**).

9. After the second transfection change medium to **full medium** and incubate the cells at 32°C and 5 % CO_2 prior to testing for transgene expression (24-72 hours).

With this procedure you can expect a transfection efficancy of ~ 40%.

Scaling Up or Down Transfections

Culture vessel	Surf. area per well[1]	Shared reagents		DNA transfection	
		Vol. of plating medium	Vol. of dilution medium[2]	DNA	Lipofectamin 2000
96-well	0.3 cm^2	100 µl	2 x 25 µl	0.4 µg	0.5 µl
24-well	2 cm^2	500 µl	2 x 50 µl	1.6 µg	2.0 µl
12-well	4 cm^2	1 ml	2x 100 µl	3.2 µg	4.0 µl
6-well	10 cm^2	2 ml	2 x 250 µl	8 µg	10 µl
60-mm	20 cm^2	5 ml	2 x 0.5 ml	16 µg	20 µl
10-cm	60 cm^2	15 ml	2 x 1.5 ml	48 µg	60 µl

[1] Surface areas may vary depending on the manufacturer.
[2] Volumes of dilution medium in Step 3a & 3b.

Full Medium:	460 ml	DMEM low Calcium (50µM)
	50 ml	FCS (Chelex treated)
	5 ml	Glutamax (100x)
	5 ml	Pyruvat (100x)
	2,5 ml	Pen/Strep
	2 ml	Adenin (250x) 45 mM
	500 µl	EGF (1000x) 10µg/ml
	500 µl	Insulin (1000x) 5 mg/ml
	250 µl	Hydroxcortison (2000x)
	5 µl	Choleratoxin (10^{-5} M)
Reduced Medium: (without Pen/Strep)	460 ml	DMEM low Calcium (50µM)
	50 ml	FCS (Chelex treated)
	5 ml	Glutamax (100x)
	5 ml	Pyruvat (100x)
	2 ml	Adenin (250x) 45 mM
	500 µl	EGF (1000x) 10µg/ml
	500 µl	Insulin (1000x) 5 mg/ml
	250 µl	Hydroxcortison (2000x)
	5 µl	Choleratoxin (10^{-5} M)
Minimal Medium: (without Pen/Strep and FCS)	460 ml	DMEM low Calcium (50µM)
	5 ml	Glutamax (100x)
	5 ml	Pyruvat (100x)
	2 ml	Adenin (250x) 45 mM
	500 µl	EGF (1000x) 10µg/ml
	500 µl	Insulin (1000x) 5 mg/ml
	250 µl	Hydroxcortison (2000x)
	5 µl	Choleratoxin (10^{-5} M)