

Testing of different transfection reagents:

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cells tested:

- A431: epidermal carcinoma, monolayer, expressing EGF-R and c-erb-B2
- HT 29: colon carcinoma, monolayer, expressing 17-1A
- CHO: chinese hamster ovary
- BHK: baby hamster kidney,
- A293: human transformed kidney,

test in 12 well plates with 8×10^4 cells/ml/well resulting in 50% confluency after one night

transfection substances tested:

- Dendrimer S
- METAFECTENE
- Lipid L

working procedures:

1,5 µg pcDNA3.1-mH-lacZ each

☞☞Dendrimer S:

0,75 µl = 1,5 µg DNA in 75 µl serumfree medium mixed with 5; 7,5; 10 µl Superfect

10 min at rt

add serum containing medium up to 400 µl and incubate for 5 h at 37°C, 5% CO₂

aspirate the content of the wells and add 1 ml serumcontaining medium for o/n incubation

☞☞METAFECTENE:

0,75 µl = 1,5 µg DNA in 30 µl serumfree medium mixed with 2,5; 5; 7,5; 10 µl

compound B in 30 µl serumfree medium

15 min at rt

add serum containing medium up to 400 µl and incubate for 5h at 37°C, 5% CO₂

add 600 µl serum containing medium for o/n incubation

☞☞Lipid L:

0,75 µl = 1,5 µg DNA in 75 µl serumfree medium mixed with 2; 7,5; 12; 18 µl Lipid L in
75 µl serumfree medium

20 min at RT.

add serumfree medium up to 400 µl and incubate for 5 h at 37°C, 5% CO₂

add 600 µl serum containing medium for o/n incubation

the next day the wells were washed with 1 ml PBS and stained with X-gal, counting of stained cells

conclusion resulting from this single test (crude data see next page):

METAFECTENE is over all less toxic than the other reagents.

METAFECTENE seems to be the best transfection reagent for all tested cell lines.

results (% of blue cells):

A293								
Dendrimer S	5µl	79% tox	METAFECTENE	2,5 µl	31%	Lipid L	2 µl	29%
	7,5 µl	71% tox		5 µl	85%		7,5 µl	41% tox
	10 µl	42% tox		7,5 µl	86%		12 µl	57% tox
				10 µl	89%		18 µl	36% tox

A431								
Dendrimer S	5µl	15%	METAFECTENE	2,5 µl	2%	Lipid L	2 µl	0,5%
	7,5 µl	15% tox		5 µl	11%		7,5 µl	3%
	10 µl	16% tox		7,5 µl	13%		12 µl	2,5% tox
				10 µl	17%		18 µl	0,7% tox

HT 29								
Dendrimer S	5µl	1%	METAFECTENE	2,5 µl	0,5%	Lipid L	2 µl	0,2%
	7,5 µl	0,3% tox		5 µl	0,6%		7,5 µl	0%
	10 µl	0,8% tox		7,5 µl	0,6%		12 µl	0,1% tox
				10 µl	0,7%		18 µl	0% tox

CHO								
Dendrimer S	5µl	71%	METAFECTENE	2,5 µl	65%	Lipid L	2 µl	47%
	7,5 µl	75%		5 µl	77%		7,5 µl	2% tox
	10 µl	63% tox		7,5 µl	81%		12 µl	3% tox
				10 µl	91%		18 µl	0,2% tox

BHK								
Dendrimer S	5µl	86%	METAFECTENE	2,5 µl	59%	Lipid L	2 µl	12%
	7,5 µl	83% tox		5 µl	93%		7,5 µl	89% tox
	10 µl	78% tox		7,5 µl	95%		12 µl	7% tox
				10 µl	94% tox		18 µl	3% tox