DNA transfection of Neuro2A, MEF wild type and MEF HSF1 KO using « Biontex K2 Transfection system »

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Cell culture

MEF cells (wild type and KO HSF1) and Neuro2A were cultured in antibiotic-free High glucose Dubelcco's modified eagle medium (Gibco) containing 10% fetal calf serum. Cells were plate in 12 well plates in 1 ml media (Neuro2A) or in 24 well plates in 0.5 ml media 24h before transfection. The transfection was performed when cells reached 80-90% of confluency

Cell transfection

Cells were treated with K2® Multiplier, 2 hours before DNA transfection. For this **K2® Multiplier** was dripped slowly onto the medium and mixed by gently swaying the dishes. **K2® Transfection Reagent** was mixed with Opti-MEM® Reduced Serum Medium (life technologies) and left on room temperature during preparation of the DNA. Plasmid-DNA encoding GFP (EGFP-C2) was mixed with Opti-MEM. DNA solution was added to the solution containing the K2® Transfection reagent (not the other way around) and mixed by inverting the tubes, followed by 15 minutes incubation at room temperature. Transfection solution was applied to cells by slow dropwise addition to the medium followed by gently swaying the dishes to achieve mixing. Transfections were incubated at 37C and 5% CO2 for 24 hours. Transfection efficiency was estimated by fluorescence microscopy. **Accurate reagent amounts are display in the table below :**

MEFKOHSF1(r1:2)

Dish sizes	DMEM	K2multiplier	K2 transfection agent	OPTIMEM/ K2 transfection agent	DNA(ug)	OPTIMEM for DNA	Mix vol final
24 well*3	0.5ml	5ul	6ul	150ul	3 ug	150 ul	300ul

DNA Final (ug)	vol Mix/puit 1	vol Mix/puit 2	vol Mix/puit 3
0.6	60ul		
0.8		80ul	
1			100ul

MEFWT(r1:5)

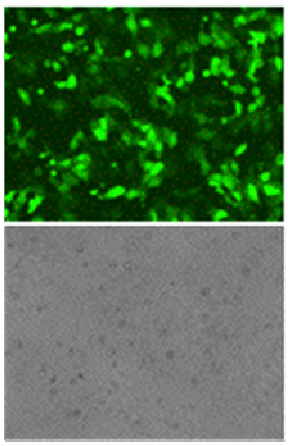
Dish sizes	DMEM	K2multiplier	K2 transfection agent	OPTIMEM/ K2 transfection agent	DNA(ug)	OPTIMEM for DNA	Mix vol final
24 well*1	0.5ml	5ul	4ul	30ul	0.8 ug	30 ul	60ul

Neuro2A (r1:2)

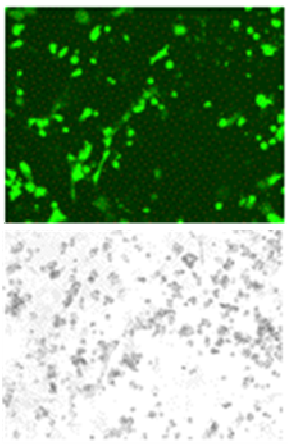
Dish sizes	DMEM	K2multiplier	K2 transfection agent	OPTIMEM/ K2 transfection agent	DNA(ug)	OPTIMEM for DNA	Mix vol final
12 well*1	1 ml	10 ul	3ul	60ul	1.5 ug	60 ul	120ul

24 wells

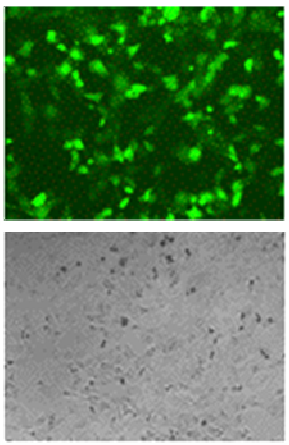
1:2 /0,4ug



1:2 /0,8ug



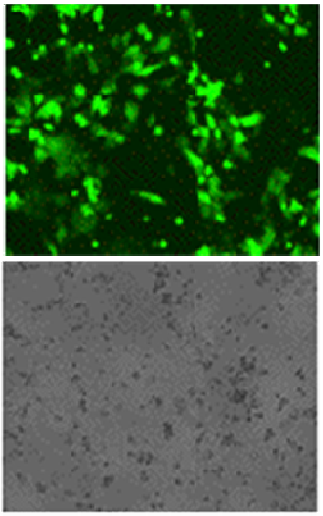
1:2 /1 ug



KO MEF HSF1

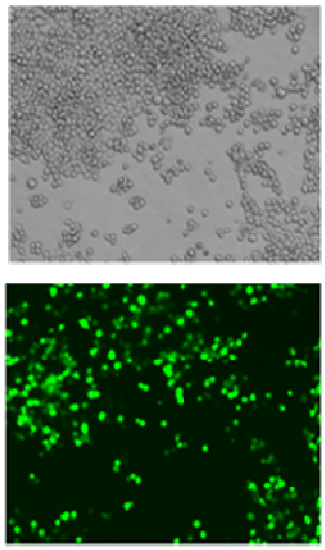
WT MEF

1:5 /0,8ug



12 wells

Neuro2A 1:2/1,5ug



Conclusions :

From all the previously tested reagents, the K2 ® transfection system is the first one able that transfect with High efficiency the MEF HSF1-KO cell line without inducing too much cell death. Good transfection efficiency was also observed in both MEF-WT and Neuro-2A cell lines.