Application Note:

Title of Experiment: Comparison of Metafectene EASY / Lipofectamine / Lipofectamine 2000

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Introduction:

The purpose of the experiment was to compare the efficiencies and the cytotoxic effect of Metafectene EASY, Lipofectamine and Lipofectamine 2000. The experiment was conducted by two different researchers. 3T3 cells were transfected by H2B-GFP and survival rates and transfection efficiency was compared between the different treatments. The H2B-GFP construct was selected as our previous experiments show that this construct is well tolerated by cells and does not exhibit any cytotoxic effect by itself. 3T3 A31 cell line was used throughout the experiment.

Materials and methods:

Three different transfection protocols were compared – Metafectene EASY, Lipofectamine and Lipofectamine 2000. The incubation times with the different reagents were set to 8h and 24h – after the incubation period, the cells were washed and culture medium was exchanged or fresh medium was added. The cells were incubated in antibiotics free media prior to transfection (DMEM supplemented with 10% Fetal Calf Serum and 2mM glutamine). The cells used were 3T3 A31 mouse fibroblast cells (ECACC 86110401). Plasmid used was H2B - pAcGFP-N1 (Clontech).

Experimental Procedures / Transfection Protocol:

3T3 cells were treated by different transfection reagents for either 8h or 24h. The transfections protocols were performed as recommended by the manufacturer: DNA (μg):Metafectene EASY = 1:1, DNA (μg):Lipofectamine = 1:12.5, DNA (μg):Lipofectamine 2000 = 1:2.5. After the incubation time, media was either changed completely (Metafectene EASY, Lipofectamine 2000) or 2X serum media was added (Lipofectamine). The experiment was evaluated 48h post-transfection.

Results and Discussion:

We have tested three different transfection reagents in 3T3 A31 cells. In our hands, Lipofectamine exhibited the least cytotoxic effect on the cells. However, highly variable efficiencies were obtained with Lipofectamine in individual experiments performed by different researchers (ranging from 15% to 80%). On the other hand, both Metafectene EASY and Lipofectamine 2000 showed more cytotoxic effect as judged by the presence of cell debris and cells with abnormal nuclear morphology (Lipofectamine 2000). This effect was especially pronounced after 24h of transfection. Thus, 3T3 cells tolerate 8h transfection better than the 24h transfection period. However, Lipofectamine 2000 treatment was very inefficient in the 8h transfection experiment (although the cells showed better survival when compared to the 24h treatment). Metafectene EASY-transfected cells showed also relatively higher GFP fluorescence (subjectively). Interestingly, the Metafectene EASY transfected surviving cells also showed normal nuclear morphology which was often not the case with the other two transfection reagents (Lipofectamine, Lipofectamine 2000). Exceptionally, up to **91%** of all surviving cells exhibited GFP fluorescence after Metafectene EASY transfection (48h post-transfection).

Conclusion / Summary:

In our hands, Metafectene EASY proved to be the reagent with which most stable results were obtained. Exceptionally, up to 91% efficiency was achieved.

Appendix: Tables and/or Figures:

Cell code	Primary	Class	Species	Organ	Type	Ident.	Description	Reagent	Growth Properties	Genetic Material	Efficiency	Toxicity
3T3 A31	NO	Mammalian	Mouse	NA	Embryonic	Fibroblast	Mouse embryonic fibroblasts	Metafectene EASY	adherent	Plasmid – H2B pAcGFP-N1	70-83%	Relatively toxic
								Lipofectamine			15-80%	Low toxicity
								Lipofectamine 2000			29-36%	Relatively toxic

An example of the abnormal nuclear morphology caused by Lipofectamine/Lipofectamine 2000 – this effect was not observed with Metafectene EASY (cytotoxicity of Metafectene EASY is comparable to Lipofectamine 2000 but higher when compared to Lipofectamine).