

Application Note:

Testing Metafectene PRO for transient transfection of EGFP in MDCK cells

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Introduction:

We were looking for a nice transfection reagent to transfect MDCK cells in absence or presence of serum

Materials and methods:

MDCK cells were grown till 90 % confluency in DMEM medium +10 % foetal calf serum.

Transfections were performed in a 8 well chamber slide (Nunc) for 24 hours

Experimental procedures / transfection protocol:

Per well (0.64 cm2): Mix1:0.2 µg DNA (coding for eGFP)+ 20µl serumfree medium was mixed in eppendorf tube by pipetting up and down.

Mix2: 1 μ l metafectene+20 μ l serumfree medium was mixed in eppendorf tube by pipetting up and down.

Mix1 was added to mix2 (=mix3) and incubated at room temperature for 20 minutes

This mix3 was added to 450 μ l of serumrich or serumfree medium on the cells (90% confluent) in the well

Results and discussion+ Conclusion / summary:

We compared Metafectene Pro with icafectin 441 (eurogentec) and fugene (roche).

The transfection efficiency was much better compared to fugene for this MDCK cell line and was comparable to icafectin but icafectin is more expensive compared to Metafectene Pro so in future we plan to use Metafectene Pro. Furthermore cell morphology was better preserved when Metafectene Pro was used.

We also observed that the transfection efficiency was much better in serumrich medium compared to serumfree medium.

References: instruction manuals of the suppliers (Biontex, fugene and eurogentec) were used to determine the transfection protocols

Appendix:



Stained with DAPI to visualize the cell nuclei