METAFACTENE Pro Technical Note

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Introduction:

METAFACTENE Pro was tested for transfection efficiency and cytotoxic side effects in a clonal cell line with oligodendroglial characteristics, namely OLN93 cells (Richter-Landsberg and Heinrich, 1996) stably transfected to express the longest human isoform of tau (Goldbaum et al., 2003). In the absence of serum and antibiotics, METAFACTENE Pro forms a lipid-DNA complex, which is able to permeate the cell membrane. DNA is released into the cytoplasm by “Repulsive Membrane Acidolysis”. A new feature of this product, the so-called “Task of Prevention”, protects the genetic material from degradation in the cytoplasm.

Materials and Methods:

- Plasmid pDsRed-N1 (Clontech) carrying the genes for a fusion molecule of a small heat-shock protein (sHSP) and the red fluorescent protein DsRed
- Sterile 60mm and 100mm culture dishes
- Sterile Eppendorf tubes
- Trypsin/EDTA solution
- DMEM
- DMEM containing 0.5 % FCS and penicillin/streptomycin
- DMEM containing 10 % FCS and penicillin/streptomycin
- OLN93-t40 cells

- Transfection reagents:
  METAFACTENE Pro
  METAFACTENE
  FuGene 6 (Roche)

Experimental procedures:

1. OLN93-t40 cells were grown on culture dishes (100mm) in 10 ml DMEM supplemented with 10 % FCS and penicillin/streptomycin at 37°C and 10 % CO₂. At ~ 80 % confluency, cells were trypsinized and plated onto poly-L-lysine-coated 60 mm culture dishes at 300,000 cells per plate, and a total volume of 5 ml. Cells were incubated for 24 hours to allow cell attachment. By the time of transfection, the cells had reached an optical confluency of approximately 70 %.

2. The plasmids were thawed on ice and diluted to concentrations of 1 – 2 µg/100µl using serum- and antibiotic-free DMEM at room temperature (total volume of 300 µl, Mix A).
3. METAFACTENE Pro was brought to room temperature and diluted with DMEM to yield final concentrations of 2 or 3 µl/100µl (total volume of 300 µl, Mix B).

4. Mix A and Mix B were combined to prepare the transfection complex at the different ratios (Metafectene Pro: DNA) indicated in Table I, mixed by carefully pipetting up and down (Pipetman), and incubated for 20 min at room temperature.

5. During this 20 min incubation period, the culture medium was removed from the cells. Cells were washed once with DMEM containing 0.5 % FCS, and 5 ml of culture medium (0.5 % FCS-DMEM) were added.

6. The transfection complex was then added to the dishes in a drop-like manner, and evenly distributed by gently moving the dishes. Cells were incubated at 37°C for the indicated times.

7. At 4 hours post transfection, the medium was changed in one batch of cells, replaced with 5 ml of fresh medium (0.5 % FCS-DMEM) and incubated for further 20 hours. The other batch was incubated with the transfection complex for 24 hours.

8. At 24 hours post transfection, all cells were monitored for transfection efficiency using an Olympus IX70 microscope supplemented with both a Hoffman modulation contrast unit and an Olympus IX-FLA fluorescence unit. Transfection rate was assessed by counting cells. DsRed-positive cells were determined as per cent of total cell number. The data shown in Table I and Figure I represent the means of three independent experiments counting at least 400 cells.

9. The transfection efficiency of METAFACTENE Pro was compared to that of two other lipofectants, i.e. METAFACTENE and FuGene 6. Both reagents were used according to the manufacturer's instructions, optimized for OLN93 cells.

Results and Discussion:

Table I, and Figure I and II show that the highest transfection efficiency of OLN93 (-t40) cells using METAFACTENE Pro occurred at a lipid-DNA ratio of 3:1. The transfection efficiency was as high as 80 %. Similar results were observed after 4 hours or 24 hours incubation time with the transfection complex, and no loss of cells occurred, indicating that METAFACTENE Pro at this concentration has very little or no cytotoxic side effects. This assumption is further supported by the observation that after incubation with METAFACTENE Pro alone, cells had a healthy morphology and stress proteins were not upregulated, as indicated by Western blot analysis (data not shown). However, after incubation with high amounts of lipid-DNA mixture, some cytotoxic effects were observable (Figure II, bottom panel). Transfection efficiency in OLN93 (-t40) cells using METAFACTENE was about 50 %. However, in contrast to METAFACTENE Pro, after 24 hours cytotoxic effects were
much more prominent and resulted in a significant cell loss. FuGene 6 in this cell system did not exert cytotoxicity, but transfection efficiency reached only about 20% (Figure I).

**Summary:**

We demonstrate that high transfection efficiencies can be obtained using METAFECTENE Pro. Successful protein expression and no apparent cytotoxicity were observed. In general, in our system the following guidelines are essential when using METAFECTENE Pro to deliver plasmid DNA into cells.

**Guidelines for Transfection using METAFECTENE Pro:**

1. Cells should be plated onto PLL-coated dishes 24 hours prior to transfection.
2. Cells should be supplied with fresh 0.5% FCS-DMEM shortly before transfection to minimize serum interference.
3. For optimal results, we recommend optimizing conditions by testing the following parameters: (1) cell density; (2) amount of plasmid DNA used in the transfection complex mixture; (3) amounts of lipid-DNA mix used per plate.
4. In general, cells must be about 70% confluent at the time of transfection. However, different cell types may require different cell densities to achieve optimal transfection and protein expression.

**References:**


Appendix I: Tables and Figures

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<tr>
<th>METAFECTENE Pro [µl]</th>
<th>DNA [µg]</th>
<th>Ratio</th>
<th>Incubation time [hrs]</th>
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Table I: Transfection efficiencies using METAFECTENE Pro. Different lipid-DNA ratios and incubation times were tested.

Figure I: Transfection efficiencies of OLN-t40 cells using different concentrations of METAFECTENE Pro in comparison with two other reagents, METAFECTENE (MF) and FuGene 6 (FG).
Figure II: OLN93 (-t40) cells transfected with pDsRed-sHSP using METAFECTENE Pro, 24 hrs after transfection. In this example, medium was changed after 4 hrs of incubation. Different ratios of METAFECTENE Pro:DNA (3:2, 2:1, and 3:1) were tested. Amounts given in brackets indicate the total amount of lipid-DNA mixture.