

MetafecteneTM Pro Transfection into Hela cells protocol

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Materials:

Plasmid pEGFP-N-fusion CIP85 protein vector
Sterile 24-well tissue culture dishes
Sterile polystyrene round-bottom tubes
Glass coverslips
Opti-MEM (GIBCO)

Optimization of the transfection for Hela cells:

Hela cells were grown in a 24well culture dishes with one glass coverslip/ well in DMEM with 10% fetal bovine serum to near 50% to 70% confluency.

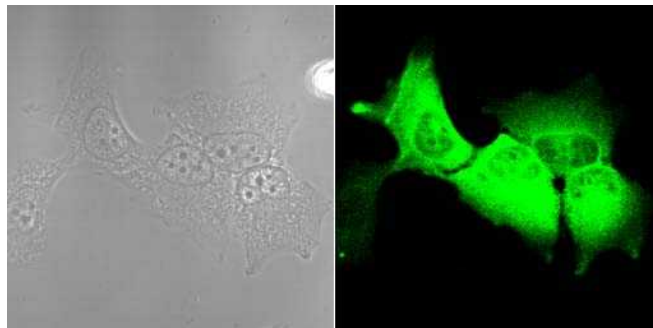
When the cells are ready for transfection with MetafecteneTM Pro, the transfection procedure was carried out as follows:

1. Prepare one polystyrene tube : add 100ul Opti-MEM medium with plasmid DNA 1ug plus MetafecteneTM Pro 1ul, gently mix.
2. The tubes were allowed to stand at room temperature for 15min min for complexes to form.
3. During this time, the cells were wished twice with fresh Opti-MEM medium, then add 100ul of Opti-MEM medium to each well. At the end of the incubation time, the DNA complex mixtures were pipetted onto the cells.
4. Incubated at 37°C incubator under 5% CO₂ more than 4hr, then change to regular medium for culture another 24 and 48h.
5. 24 and 48h post transfection starting time, the cells were either observed under fluorescent microscope or fixed with cold 80% methanol/20% acetone at -4°C for 15 min. the cells were mounted. The transfection rate was determined by counting the per cent of fluorescent cells expressing the EGFP-tagged protein under any fluorescent microscope.

Conclusions:

1. MetafecteneTM Pro has the same transfection efficiency as LipofectAMINE 200 in Hela cells.
2. MetafecteneTM Pro is less cytotoxicity and much better than the METAFECTENE reagent.

Figure Legend: Hela cells were transfected with a plasmid encoding for a dynamin-associated EGFP fusion protein using the MetafecteneTM Pro reagent for 24hr. according to the conditions described in this study.



A) Phase contrast image of the cells

B) Fluorescent image of the transfected cells shows that cells express the GFP fusion protein.