Biontex

Metafectene Technical Note

Dr. Andrea Blöchl, Nina Hambruch

Biochemie II, Fakultät Chemie, Ruhr-Universität Bochum, 44790 Bochum

Optimization of the transfection rate in neuroblastoma cells NS20-Y and SHSY-5Y

Mouse NS20-Y and human SHSY-5Y are adhesive neuroblastoma cell lines. Both cell lines form extensive processes in culture and tend to grow in clusters. Cell cycles of these cell lines are rather slow (60 h for NS20-Y and about 40h for SHSY-5Y).

Cells were grown in NUNC-25 ml flasks with DMEM (4500g/l glucose), supplemented with 10% fetal calf serum (FCS) and 2 mM glutamine. The cultures were splitted 1:2 all four to five days using PBS (without $Ca^{2+}$and Mg^{2+}) and 1 mM EDTA for detaching. Prior to transfection cells were seeded into 24-well plates at a density of 10^5 cells/well 16-20h. We estimated the maximal confluence of cells at the time of transfection to about 80%.

To determine optimal conditions for transfection, different concentrations of Metafectene and DNA were tested as shown in tables 1 and 2. Metafectene and DNA (expression-plasmid with enhanced green fluorescence protein, EGFP) were diluted in 50 µl DMEM without FCS as indicated in the tables, mixed and incubated at room temperature to allow the formation of the DNA-Metafectene complex. The cells were transferred into serum-free, confined B18-medium (Brewer, G.J., Cotman, C.W., Brain Res. 494, 65ff; 1989; 500µl/well) and the DNA-Metafectene mixture was added to the medium. Cells were incubated for 6h at 37°C and 10% CO_2. The medium was then exchanged with DMEM / 10% FCS (PAA,"clone") and cells were grown for additional 30h. Finally, the expression of EGFP within the cells was analyzed with the inverse microscope IM2 (Zeiss) by counting the number of fluorescent cells among a total of about 500 cells per well. The calculated percentage of fluorescent cells in the culture is given in tables 1 and 2. Since the cells formed clusters (particularly NS20-Y) exact cell counting often proved to be impossible and careful estimations had to be used.

In comparison with Metafectene electroporation and Ca_2PO_4-precipitation were used; the transfection rate with these transfection methods was about 10%.

To evaluate the effect of cell division on the transfection rate, transfection was performed at different intervals after culture splitting. The cells were transfected directly or 24, 36, or 48h after spliting. Under optimal conditions (see tables), with NS20-Y cells a slight elevation of transfection rate to about 80% could be observed if cells were transfected immediately after seeding; transfection at 36h after splitting resulted in a transfection rate of about 30 %. With SHSY-5Y cells the transfection rate increased to about 50% if cells were transfected directly after seeding, and was lowest at 36h and 48h.

Generally we observed at least about 10% cell death during transfection, dependant on concentration and incubation time of Metafectene. Using more than about 2-3 µl Metafectene increased cell death to more than 70%.

Discussion

Due to slow cell proliferation, transfection of neuroblastoma cells is rather difficult. Up to now, we obtained the best transfection rates (about 10%) with Ca_2PO_4-precipitation; such rates, however, are intolerable for most biochemical studies. Using Metafectene, transfection rates of 80% with NS20-Y cells and 50% with SHSY-5Y cells could be observed when transfection was performed directly after culture splitting. Since splitting often induces cell division and cells usually prefer uptake of DNA during S- and M-phase of the cell cycle, the elevated uptake directly after splitting might be due to a partial synchronization of cell division.

The optimal ratio of DNA/Metafectene is 1:4 (NS20-Y) resp. 1:8 (SHSY-5Y); the amount of Metafectene should not exceed 2µl, since higher concentrations seem to be toxic. The toxicity of transfection could be further lowered by changing the medium 6h after transfection and using endotoxin-free sera and DNA preparations.

Transfection was performed in serum-free B18-medium (containing BSA). FCS in the medium lowered the transfection rate slightly (by about 10%). Sera seem to disturb the formation of DNA/Metafectene complexes.

Table 1: Transfection-rate of NS20-Y cells using different amounts of Metafectene and DNA; transfection 16-20h after culture splitting

Metafectene (µl)	DNA (µg)	DNA/Metafectene	% transfected cells
0.5	0.25	1:2	10
1	**0.25**	**1:4**	**76****
2	0.25	1:8	33
3	0.25	1:12	40
1	0.5	1:2	25
2	0.5	1:4	10
4	0.5	1:8	10*
6	0.5	1:12	/*
2	1	1:2	25
4	1	1:4	10*
6	1	1:6	25*
8	1	1:8	/*

*cell death over 70%

**optimization by transfection directly after culture splitting: 80%

Table 2: Transfection-rate of SHSY-5Y cells using different amounts of Metafectene and DNA; transfection 16-20h after culture splitting

Metafectene (µl)	DNA (µg)	DNA/Metafectene	% transfected cells
0.5	0.25	1:2	10
1	0.25	1:4	20
2	**0.25**	**1:8**	**33****
3	0.25	1:12	10*
1	0.5	1:2	15
2	0.5	1:4	20
4	0.5	1:8	10*
6	0.5	1:12	5*
2	1	1:2	20
4	1	1:4	25
6	1	1:6	10*
8	1	1:8	5*

*cell death over 70%

**optimization by transfection directly after culture splitting: 50%