

## Transfection optimisation of HT-22 in 24 well- and 96 well plates.

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In our studies we used the immortalized mouse hippocampal cell line HT-22, which is a well characterized model to study the effects of oxidative stress in cell culture. HT-22 is an adherent growing cell line, cultivated in DMEM supplemented with 10% FBS (heat inactivated) and 1% of each sodium pyruvate, L-glutamine and penicillin/strepromycin. To achieve comfortable transfection efficiencies it is absolute necessary to use a pre-culture in exponential growth.

**24 well plate:** depending on plate consistence and supplier it might be necessary to coat the plates with gelatine or other products. 24h before transfection **30.000** cells/well were seeded in 800µl DMEM and incubated for 24h at 37°C and 5% CO<sub>2</sub>.

Transfection-reagents were prepared in 1,5ml Eppendorf-tubes.

Transfection:

Reporter DNA [µg]/	Metafectene [µl]/	Medium change	Efficiency	Toxicity
DMEM [µl]	DMEM [µl]	after 4 h	[%]	[%]
0,8 / 30	3,0 / 50	yes	50	0
1,0 / 30	3,0 / 50	yes	50-60	0
1,3 / 30	4,0 / 50	yes	60-70	0

Cell viability was determined using light microscopy. Transfection efficiencies were measured 40h after transfection in a fluorometric read out.

**Note**: Medium change does not improve the transfection efficiencies, but due to expression periods up to 40h it enhances the cell's living conditions.

## **96 well plate:** depending on plate consistence and supplier it might be necessary to coat the plates with gelatine or other products. 24h before transfection **2000** cells/well were seeded in 100μl DMEM and incubated for 24h at 37°C and 5% CO<sub>2</sub>.

Reporter DNA [ng]/	Metafectene [µl]/	Medium change	Efficiency	Toxicity
DMEM [µl]	DMEM [µl]	after 24 h	[%]	[%]
50 / 30	0,93 / 30	yes	1	0
70 / 30	0,93 / 30	yes	1	0
100 / 30	0,93 / 30	yes	10	0
150 / 30	0,93 / 30	yes	50	0
200 / 30	0,93 / 30	yes	60	0
250 / 30	0,93 / 30	yes	60-70	10
300 / 30	0,93 / 30	yes	30	80

Transfection- reagents were prepared in 96 well plates.

Cell viability was determined using light microscopy. Transfection efficiencies were measured 40h after transfection in a fluorometric read out.

- **Note**: In comparison to transfection in 24 well plates, transfection efficiencies are decreased if the medium is changed 4h post-transfection. In general Metafectene shows no toxic side effects on HT-22 in 96 well/plates. Due to the age of the cells and the length of the expression period it nevertheless might be necessary to change the medium 24h post-transfection.
- **Note:** The data shown in these tables were optimized for the mentioned cell-numbers and expression periods. Shortening the expression period or increasing the number of cells will lead to different transfection efficiencies and might require different reagent combinations.