

Transfection of P815 and EL4 using NIMT[®]FeOfection|YELLOW

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Introduction

Murine suspension cells, P815 (Murine Mastocytoma cells, derived from white mouse mast cells) and EL4 (Mouse lymphoma cell line, derived from black mouse lymphocytes) were transfected with DNA using NIMT[®]FeOfection|YELLOW. Two to five days after transfection cell supernatants were analyzed for soluble Hepatitis B antigen (HBsAg).

Transfection

On the day of transfection, 25 000 cells were seeded in a 96-well plate in 100 µl growth media. The stock solution of NIMT[®]FeOfection|YELLOW was diluted 5 times in sterile double distilled (dd) H₂O and DNA was diluted to a concentration of 200 µg/ml in sterile dd H₂O. NIMT[®] Booster was used with a ratio of 2 µl NIMT[®] Booster to 1 µg DNA.

NIMT[®]FeOfection|YELLOW was then diluted further according to table 1 and NIMT[®] Booster was added to the particles according to table 1. Finally 5 µl of the diluted DNA was added per tube (not to the Mock sample). The solutions were mixed and incubated 10-20 min in room temperature. 20 µl of the transfection solution was added to each well.

Cell supernatants were collected after 2, 4 and 5 days of incubation for analysis of HBsAg. For analysis for HBsAg a standard method was used (HBsAg ULTRA, Vidas, an automatic sandwich assay).

Table 1. The amount of particles, NIMT[®]Booster and DNA used per well in a 96-well plate.

Sample	NIMT [®] FeOfection (Diluted 5 times)	NIMT [®] Booster	H ₂ O	DNA 200 µg/ml	Final volume
1	6 µl	2 µl	7 µl	5µl	20 µl
2 (Mock)	6 µl	3 µl	11µl	-	20 µl

Results

Both P815 and EL4 were successfully transfected with NIMT[®]FeOfection|YELLOW. The analysis method is a non-quantitative method. Values >=0.1 are considered to be positive for soluble Hepatitis B Ag, see table 2.

Table 1. Results of the HBsAg assay. The assay was performed on cell supernatants 2, 4 and 5 days after transfection.

Cell Line	Days after transfection and test values		
	2 Days	4 Days	5 Days
EL4	ND	3.18	ND
P815	0.96	1.28	2.68