K2 Transfection Technical Note

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Materials: Plasmid: pHIV-dTomato;
DMEM supplement with 10% fetal calf serum and 1% antibiotics;
Cell line: U87MG (glioma).

Protocol:

$1x10^4$ or $2x10^4$ U87MG were plated in 96 well plate with 100 uL completed medium and incubated in 5% CO_2 at 37°C for 24 hrs. Subsequently, it was performed the transfections as following:

1) K2 multiplier and K2 transfection reagent were placed at room temperature and gently mixed;
2) 1 uL of K2 Multiplier was added to each well and incubated for 2 hrs;
3) Solution A: 400 ng DNA in 20 uL
   Solution B: **(1:3)** 0,6 uL K2 Transfection Reagent in 10 uL serum-free medium
   Solution B: **(1:4)** 0,8 uL K2 Transfection Reagent in 10 uL serum-free medium;
4) 10 uL of Solution A was added to Solution B and incubated at RT for 15 min.
5) 20 uL of DNA-lipid complex was added to each well. The solution was gently mixed and the cells were incubated overnight at 37°C in CO_2 incubator.
6) In the next day, the medium was replaced and transfection efficiency was analyzed in fluorescent microscopy;

RESULTS

In terms of cell viability all tested conditions were not toxic to cells.
In terms of transfection efficiency the best condition was = **1:4** and **$2x10^4$** cells
Transfection efficiency: U87MG = about **40-50%**