Application Note K2 Transfection System

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Experimental Details

Cell type: Human monocyte-derived macrophages
Cell number: $3x10^5$ cells per well
Culture vessel: 24-well plate
Culture medium: RPMI 1640 + 10% FBS + 1% Glutamax + 1% Sodium Pyruvate + 0.1% β-mercaptoethanol
Nucleic Acid: fluorescent oligonucleotide duplex (siGLO - Thermo Scientific)

Human macrophages were seeded and differentiated using 20ng/ml of M-CSF for 1 week in a 24-well plate. After differentiation, cells to be transfected with K2® Transfection Reagent were incubated with 5µl of K2® Multiplier in 600µl of culture medium and incubated for 2h.

Transfection solution was prepared in two separate vessels:

- 50 µl of serum-free medium + desired amount of RNA oligo to achieve a final concentration of 50nM or 25nM (final volume of culture medium will be 600µl)
- 50 µl of serum-free medium + 9 µl or 4.5 µl of transfection reagent

Transfection solutions were gently mixed, adding the nucleotide solution to the transfection reagent solution, and incubated for 20 min. Meanwhile, human macrophages were washed with PBS and 500 µl of culture medium was added to each well.

Following that, 100µl of the transfection solution was added to each well, mixed by gently agitating the plate, and incubated for 24h.

After 24h cells were washed with PBS, fixed with 4% PFA for 15 min then the percentage of fluorescent cells was quantified by flow cytometry.

Results

Our results show a consistently higher effective transfection of our cells using the K2® Transfection system and visual observation also reveled a lower amount of dead cells.