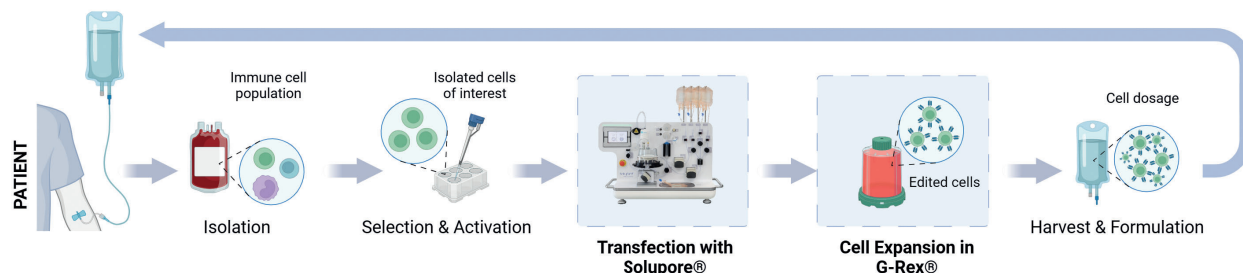
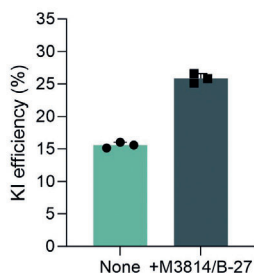


Solupore® knock-in using GenScript GenExact™ single-stranded DNA

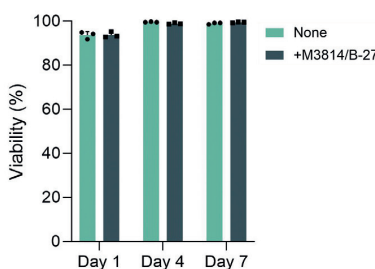
Solupore® is an automated, closed non-viral delivery platform available for cell engineering utilising a gentle and rapid physico-chemical transfection technology, which maintains superior cell health and function post-engineering. In this application, Solupore delivered a GenScript GenExact™ single-stranded DNA (ssDNA) to T-cells and achieved a 25% knock-in efficiency with excellent cell viability.



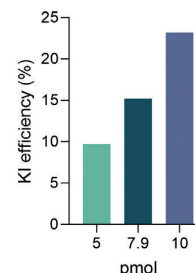
Knock-in Efficiency



Cell Viability



ssDNA Dosing



Results

Knock-in Efficiency:

Robust levels of eGFP positive CD3+ cells were observed on day 7 post-delivery of ssDNA_TRAC_EGFP_CTS by Solupore RT. Cells were cultured in G-Rex plates with and without the HDR enhancer (M3814/B-27).

Cell Viability:

High percentage of viable CD3+ cells assessed on days 1, 4 and 7 post-delivery of ssDNA_TRAC_EGFP_CTS by Solupore RT. Cells expanded up to 8-fold over 7 days.

ssDNA Dosing:

Increasing percentages of eGFP positive CD3+ cells were observed on day 7 post-delivery of ssDNA_TRAC_EGFP_CTS by Solupore RT with increasing ssDNA concentration.

Conclusion

The results demonstrate that Solupore® enables robust levels of knock-in efficiency using GenScript's GenExact™ ssDNA, while preserving exceptional cell health and viability.

Methods

Healthy donor CD3+ cells were activated in TexMACS+5% HAB with TransAct (Miltenyi Biotec) and IL-7/IL-15 (Bio-Techne; 10ng/mL & 5ng/mL respectively) for 48 hr before transfection. Cas9 RNP (TRAC; 210 pmol; 4:1 molar ratio) was complexed with ssDNA_TRAC_EGFP_CTS (37.2 pmol, 1630 nt) and incubated for 2 min at room temperature before being added to Solupore delivery solution. Six million cells were transfected using Solupore RT and the sample was split into 2 × 1E6 cells and seeded into a 24-well GRex plate. The HDR enhancer M3814 (1µM) and media supplement B-27 (1x) were added to one of the samples 24 hr before the media was changed to TexMACS+5% HAB with IL-7/IL-15 and B-27. A sample for cell count and viability (NC200) was taken on days 1, 4 and 7. Cells were harvested on day 7 and assessed for knock-in efficiency (using eGFP expression) by flow cytometry.

