Protocol 1040

GEMINI & ECM® 830 ELECTROPORATION PROTOCOL

Cell Line: Primary Human CD4 and CD8 T lymphocytes (CAR T cells)

Application or Transfectant: CRISPR - Cas9 mRNA

Electrode: 2 mm gap cuvette Item # 45-0125

Cell Preparation:

Primary human CD4 and CD8 T cells were isolated from healthy volunteer donors following leukapheresis. The T cells were washed three times with OPTI-MEM and re-suspended in OPTI-MEM (Invitrogen) at a final concentration of 1 to 3 × 10^8 cells/ml. The recommended electroporation buffer with the current ECM 830 model is BTXpress high performance buffer. Electroporation of CRISPR reagents with one-shot CAR or CAR T cells was performed with a BTX ECM 830 electroporator.

Square Wave Electroporation Settings:

Set Voltage:	360 V
Set Pulse Length:	1 ms
Set Number of Pulses:	1
Desired Field Strength:	1800 V/cm

Electroporation Procedure:

Electrode Gap:	2 mm gap cuvette, Item # 45-0125
Total Sample Volume:	100 µl
Number of Cells:	1 to 3 × 10^8 cells/ml × 10^6 PBMCs/ml
Electroporation Buffer:	Opti-MEM or BTXpress High Performance Electroporation Solution (Item # 45-0802)
Amount of Transfectant:	20 µg cas9 RNA (per TCR chain)
Pulse:	Press the Go icon or the Start button to activate the automatic charge and pulse sequence
Post Treatment:	The cells were immediately placed in 2 ml of pre-warmed culture media and cultured in the presence of IL-2 (100 IU/ml) at 37°C and 5% CO_2.

Results:

The research group developed an easy-to-use immunoassay kit to control T cell bioassays by transfecting RNA-encoded TERS (TCR-engineered reference samples) into primary lymphocytes. High transfection efficiencies of up to 97.3% antigen-specific CD8+ T cells and high viability up to 96.0% were obtained with the BTX ECM 830 Electroporation System.

Efficiency: 97.3% antigen-specific CD8+ T cells

Viability: 96%

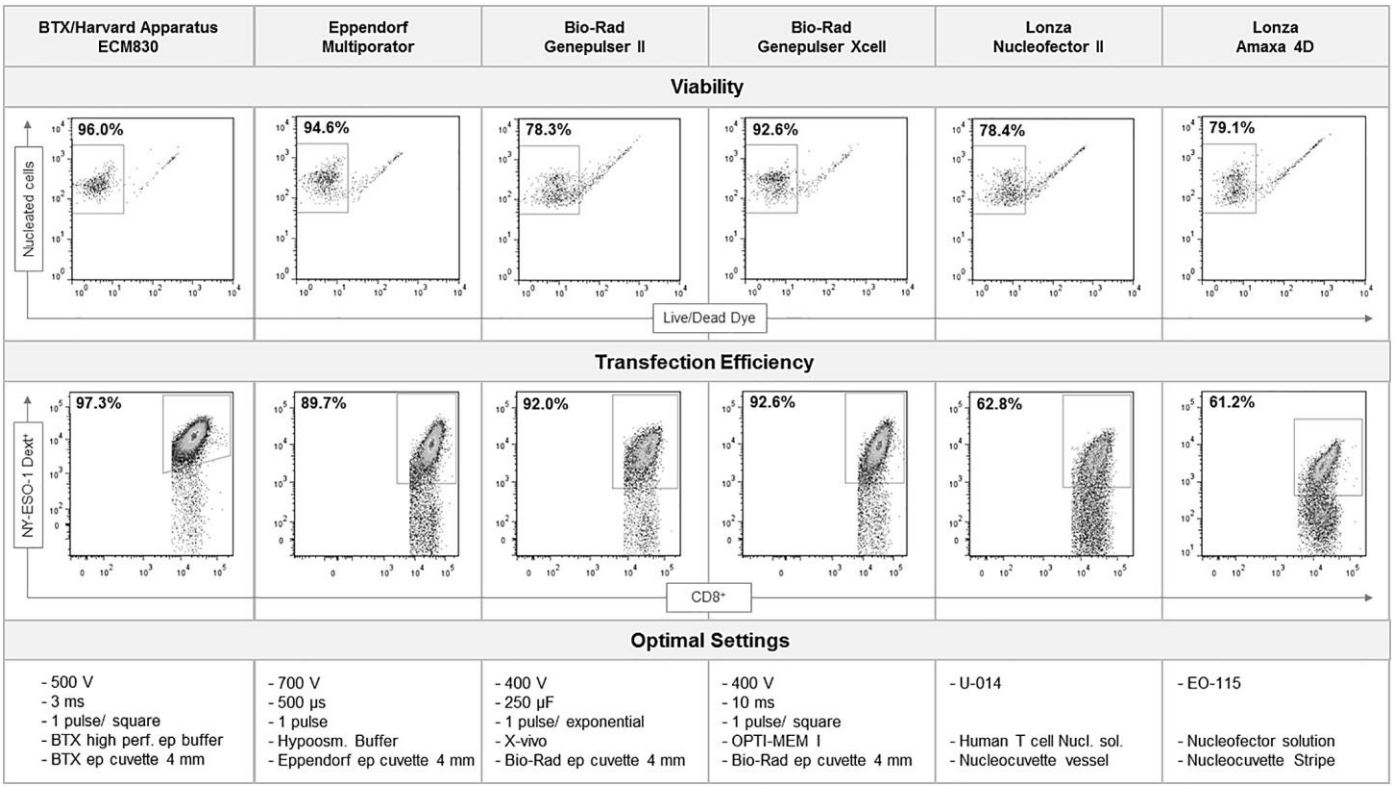

Figure 2 from Bidmon et al., Optimized electroporation settings for six commonly used electroporation devices including: the BTX ECM 830 from Harvard Bioscience; the Eppendorf – Multiporator; the Bio-Rad - Genepulser II and Xcell; and, the Lonza - Nucleofector II and Amaxa 4D. The BTX ECM 830 achieved TCR expression levels higher than the benchmark of > 80% TCR+ of CD8+ and a viability higher than the benchmark > 90% viability.

References:

Bidmon N et al., Development of an RNA-based kit for easy generation of TCR-engineered lymphocytes to control T-cell assay performance. J. Immunol. Methods 2018; 458: 74–82.

Compiled by: Radhika Dixit, PhD, BTX Applications Scientist, 10/17/2018