GENOME EDITING OF HUMAN PRIMARY T CELLS

Using the ArciTect™ CRISPR-Cas9 System

CRISPR-Cas9, an RNA-guided genome editing technology, is revolutionizing cell biology due to the ease and efficiency by which it enables the genetic manipulation of mammalian cells. Through the targeted modification of specific genes or regulatory regions, researchers can now rapidly generate precise genetic models to study normal and diseased cell physiology.

Early attempts to apply CRISPR-Cas9 for genome editing in human primary T cells used either viral vectors[1,2] or plasmids[3,4] for Cas9 and gRNA expression, resulting in low targeting efficiency and high toxicity. More recently, ribonucleoprotein (RNP)-based CRISPR-Cas9 expression systems have achieved high efficacy across a number of targets.[5-7] ArciTect™ is an RNP-based system that supports genome editing of human primary T cells. Use our optimized protocol (Document #27155) to achieve high efficiency genome editing and robust post-editing expansion of T cells.

Why Use ArciTect™?

CUSTOMIZABLE. Design crRNA to target your sequence of interest.

FLEXIBLE. Meet your specific genome editing needs with multiple variations of Cas9.

RAPID. Immediate activity of CRISPR-Cas9 RNP.

REDUCED OFF-TARGET EFFECTS. Minimize potential off-target cutting with timely degradation of the RNP complex.

COMPLETE WORKFLOW. Obtain everything you need to culture, edit, and expand human primary T cells.

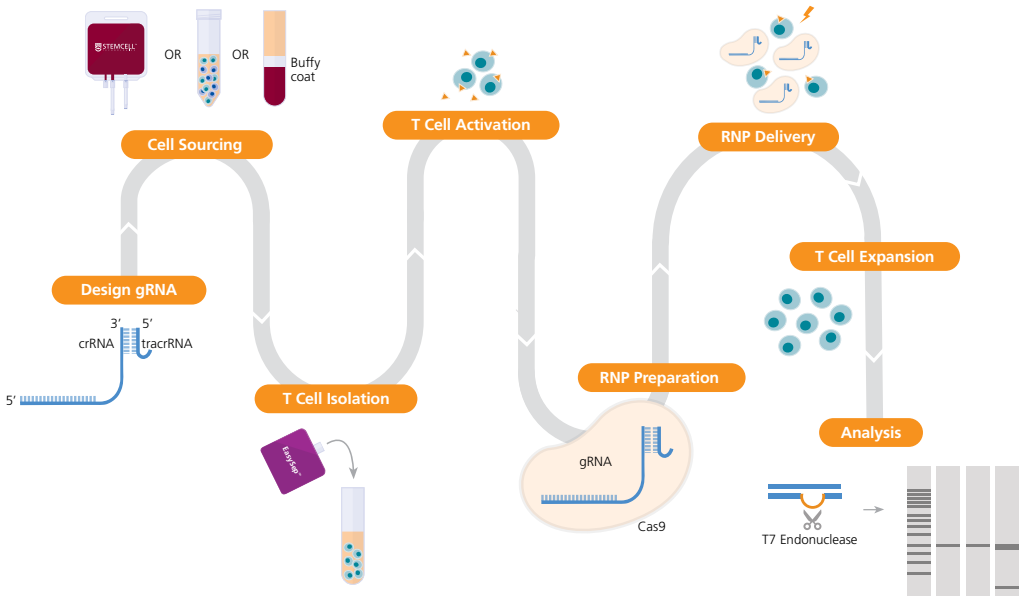

Figure 1. Experimental Workflow for T Cell Genome Editing

The guide RNA sequence is designed once a target locus for editing is identified. Human primary T cells can be sourced from STEMCELL or isolated from a number of sources, including whole blood, buffy coat, washed leukaphoresis samples, or peripheral blood mononuclear cells (PBMCs), using STEMCELL column-free cell separation technology, including EasySep™. Next, T cells are activated with either ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971) or ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Catalog #10970). The ArciTect™ CRISPR-Cas9 RNP is then prepared and delivered into T cells using electroporation, and cells are expanded in ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981). Editing efficiency can be analyzed using the ArciTect™ T7 Endonuclease I Kit or flow cytometry, if the experimental design permits. Orange triangles indicate ImmunoCult™ Human T Cell Activator.

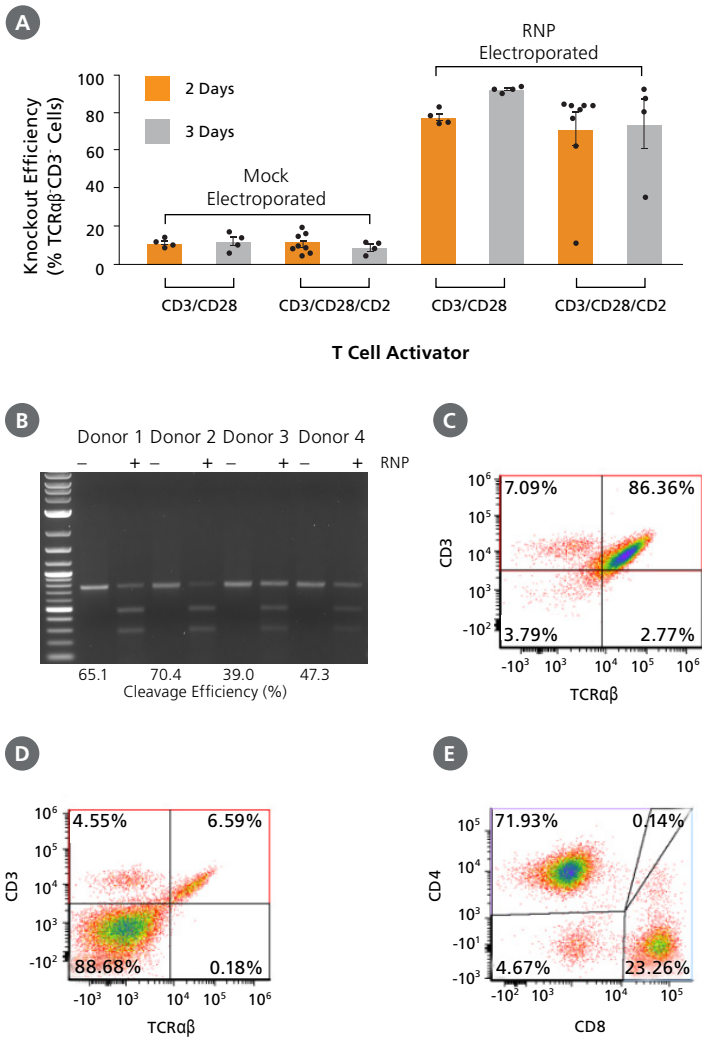

Figure 2. High Efficiency TRAC Knockout Across Activation Conditions and Dynamics, Using ArciTect™

Human T cells were activated with ImmunoCult™ Human CD3/CD28 or CD3/CD28/CD2 T Cell Activators for 2 or 3 days and the cells were electroporated with ArciTect™ RNP-complexes to knockout the T cell receptor (TCR) alpha constant (TRAC) locus. (A) Knockout efficiency was assessed 2 or 3 days after electroporation by flow cytometry analysis of TCRαβ and CD3 expression. 3 day activation with ImmunoCult™ Human CD2/CD28 T Cell Activator showed highest editing efficiency. (B) Genome editing (cleavage) efficiency was assessed at 48 hours post electroporation in human T cells activated with ImmunoCult™ Human CD3/CD28 T Cell Activator for 3 days using the ArciTect™ T7 Endonuclease I Kit. Mock electroporated: - RNP; RNP electroporated: +RNP. (C,D) Representative flow cytometry plots of control (mock electroporated, C) and edited T cells (RNP electroporated, D). (E) Edited T cells retained expression of CD4 and CD8.

For complete instructions, refer to Technical Bulletin: Genome Editing of Human Primary T Cells (Document #27155).

Comparison of Different CRISPR-Cas9 Methods[8]

Cas9	DNA	mRNA	Protein
Efficiency	+	++	+++
Specificity	+	++	+++
Degradation	> 72 hours	~ 72 hours	< 72 hours
Off-Target Cutting	High	Moderate	Low

Product Information

Product	Size	Catalog #
ArciTect™ Cas9 Nuclease	100 µg 300 µg	76002 76004
ArciTect™ Cas9-eGFP Nuclease	100 µg	76006
ArciTect™ Cas9 Nickase	100 µg	76009
ArciTect™ crRNA	2 nmol 10 nmol 20 nmol	76010 76011 76012
ArciTect™ tracrRNA Kit	5 nmol Kit 10 nmol Kit 20 nmol Kit	76017 76018 76019
ArciTect™ Annealing Buffer (5X)	1 mL	76020
ArciTect™ Human HPRT Positive Control Kit	1 Kit	76013
ArciTect™ T7 Endonuclease I Kit	25 Reactions 125 Reactions	76021 76022
ArciTect™ High-Fidelity DNA Polymerase Kit	500 Reactions	76026

References

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2. Li C et al. (2015) Inhibition of HIV-1 infection of primary CD4+ T-cells by gene editing of CCR5 using adenovirus-delivered CRISPR/Cas9. J Gen Virol 96:2381–93.
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4. Su S et al. (2016) CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients. Sci Rep 6: 20070.
5. Schumann K et al. (2015) Generation of knock-in primary human T cells using Cas9 ribonucleoprotein. Proc Natl Acad Sci USA 112: 10437–42.
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8. Liang X et al. (2015) Rapid and highly efficient mammalian cell engineering via Cas9 protein transfection. J Biotechnol 208: 44–53.