Engineered Primary T cells Quick Start Guide

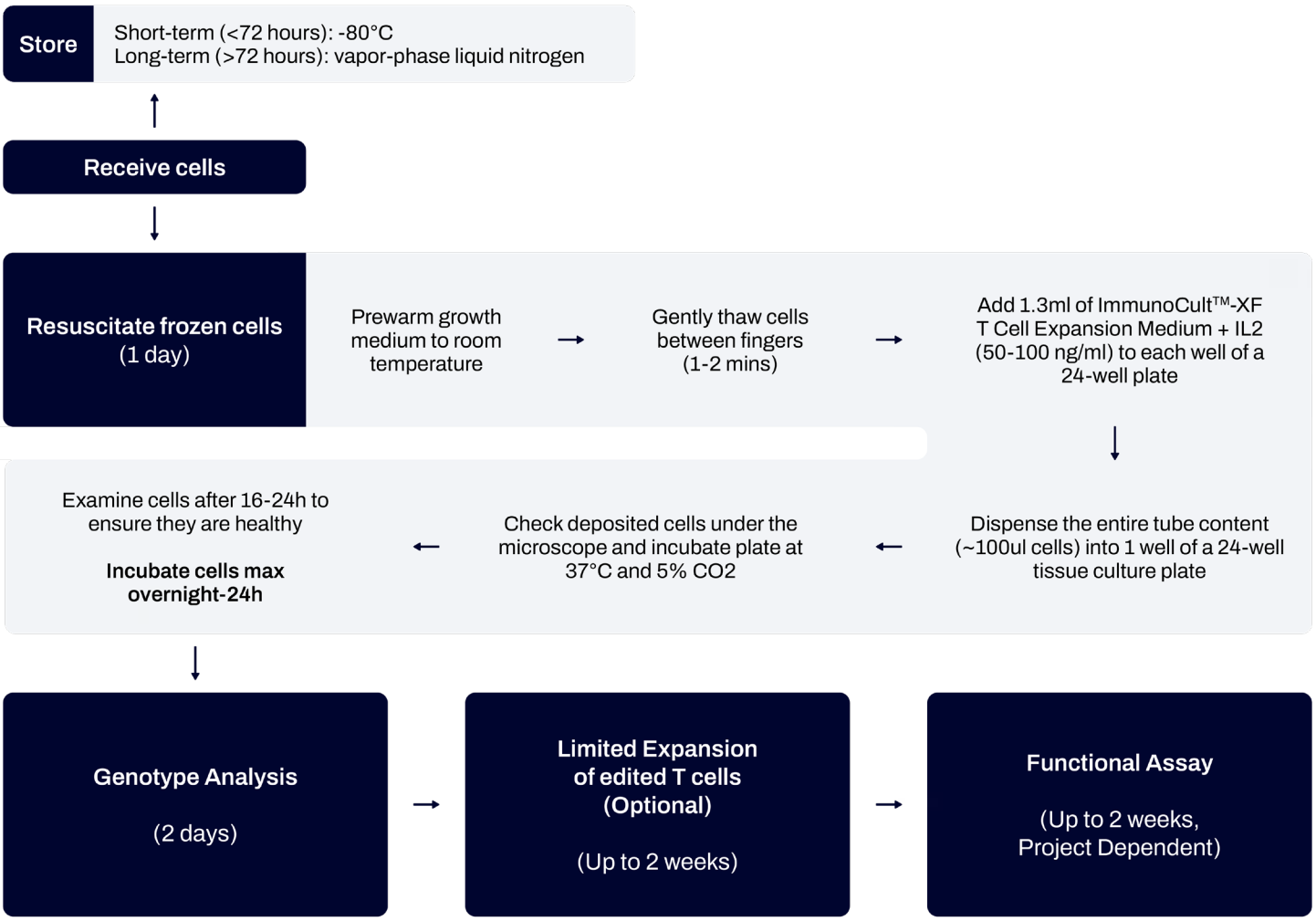
Thank you for choosing EditCo Knockout T-cells Pools for your CRISPR experiment!

Your knockout cell pool was created using high-quality synthetic multi-guide sgRNA and SpCas9 transfected as RNPs. Multi-guide technology ensures high editing efficiency without the use of any selection markers that can negatively affect cell biology. In this guide, we include important information on how to store, resuscitate, and culture your cell pool. For additional details about your order, please reference the corresponding QC report.

Important Considerations

- **Please note that if the edited T cells are not recovered using the recommended conditions described in this Quick Start Guide, we cannot guarantee the viability and healthy recovery of the cells.** Cell density plays a key role in recovering the cells post-thaw. Please carefully follow the resuspension and plating instructions included in Step 2.
- **No antibiotic selection genes** were introduced when producing your cells. If desired, broad-spectrum antibiotics/antimycotics can be added to the medium to prevent contamination.
- **Cells come frozen in the following freezing media: CryoStor® CS10 Freeze Media.** This medium contains 10% DMSO, is a ready-to-use medium, and no additional supplements are needed. Please use this freezing media if you wish to make frozen stocks.
- **Do not wash or centrifuge cells after thawing the cells to remove the freezing medium.** Thawed cells should be deposited directly into complete culture media to dilute the freezing medium and maximize cell recovery.
- **We highly recommend confirming the genotype of cells and their protein expression or performing functional assays within 24 hours of thawing the cells.** These primary immune cells have a finite lifespan and the cell pool population's genotype can change over time if the knockout editing impacts the cell growth dynamics.
- Please refer to our Sample QC report if you have any questions regarding the information included in your QC report.

Workflow Schematic



Materials Required

Name
Complete T cell growth medium (StemCell Technologies ImmunoCult™-XF T Cell Expansion Medium. Cat# 10981)*
Recombinant Human Interleukin 2 (IL2) (Peprotech Cat #200-02)
ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Catalog # 10970)
CryoStor® CS10 Freeze Media (StemCell Technologies # 100-1061)
24-well G-Rex® plate (Wilson Wolf cat# 80192M)
70% isopropyl alcohol or 70% ethanol (in a spray bottle)
Kimwipes
Sterile 24-well tissue culture plate(s) (Multiple vendors e.g, Thermo Fisher Scientific)
Sterile pipettes (Multiple vendors e.g, Thermo Fisher Scientific)
Microscope for monitoring cell recovery and phenotype
Biosafety cabinet at appropriate containment level
Cell incubator

*EditCo has tested and recommends StemCell Technologies ImmunoCult™-XF T Cell Expansion Medium supplemented with Interleukin-2 (IL2). Other media formulations have not been directly tested against the recommended medium. Please contact the Scientific Support Team at technicalsupport@editco.bio if you have questions on which medium to use.

Step 1. Receive Cells & Place in Storage

1. EditCo cells ship frozen. Knockout T-cell Pools are provided in 1.1 ml Micronic tubes. Each tube contains ~100 µl of cell suspension. After receiving cells, immediately place them in storage. Cells should be frozen at –80°C for short-term storage (≤72 hours) and placed in vapor-phase liquid nitrogen for longer-term storage (>72 hours). With appropriate storage, cells can be kept for as long as needed.

2. If desired, cells can be thawed immediately after arrival. Please follow Step 2. We highly recommend confirming the genotype of cells or performing functional assays within 24 hours of thawing the cells.

Step 2. Resuscitate the Frozen Cells

Note: Ensure all steps are completed using aseptic technique to prevent contamination.

1. Pre-warm complete growth medium to room temperature (EditCo has tested and recommends StemCell Technologies ImmunoCult™-XF T Cell Expansion Medium, cat# 10981, supplemented with Interleukin-2 (IL2) from Peprotech (cat# 200-02) at 50-100 ng/ml). Please contact the Scientific Support Team at technicalsupport@editco.bio if you have questions on what medium to use.

2. Prepare a 24-well tissue culture plate (label with the cell line name, passage number, and date) and add 1.3 ml of complete growth medium to one well.

3. Remove a tube containing frozen cells from cold storage and gently wipe the tube exterior with a Kimwipe saturated with 70% ethanol or isopropyl alcohol before placing the tube into the biosafety cabinet.

4. Gently roll the tube between fingers until completely thawed (1-2 minutes).

5. Set the volume of the corresponding micropipette to 110 µl, depress the pipette plunger, position it slightly above the bottom of the tube, and slowly draw up cells.

   NOTE: Avoid touching the bottom of the vial to prevent shearing of cells.

6. Dispense the 100 µl of cells into the well of the 24-well plate containing the complete growth medium.

7. Check the well for deposited cells under a microscope. Shortly after seeding, cells should appear as shown in Figure 1 below. Transfer the tissue culture plate to an incubator set at 37°C and 5% CO2.

   NOTE: We recommend avoiding incubating longer than overnight or 24 hours under these conditions. Cells can be expanded. **If you would like to culture the cells > 24 hours please follow step 4.**

8. Examine the cells after 16 - 24 hours to ensure they are healthy (e.g., forming typical clusters of actively dividing cells and absence of contamination). Cell clumping is part of T cell biology when these cells are activated via T cell Receptor (**Figure 1**).

9. (**Optional**) Trypan blue dye exclusion can be used as a method to determine cell viability and counting. If instructions are followed, viability is expected to be >70% at 24 hours after thawing using this method. Please refer to this article for instructions on how to perform Trypan blue dye exclusion on your Knockout T-cell Pools. Please note different cell viability and counting methods can lead to different results.

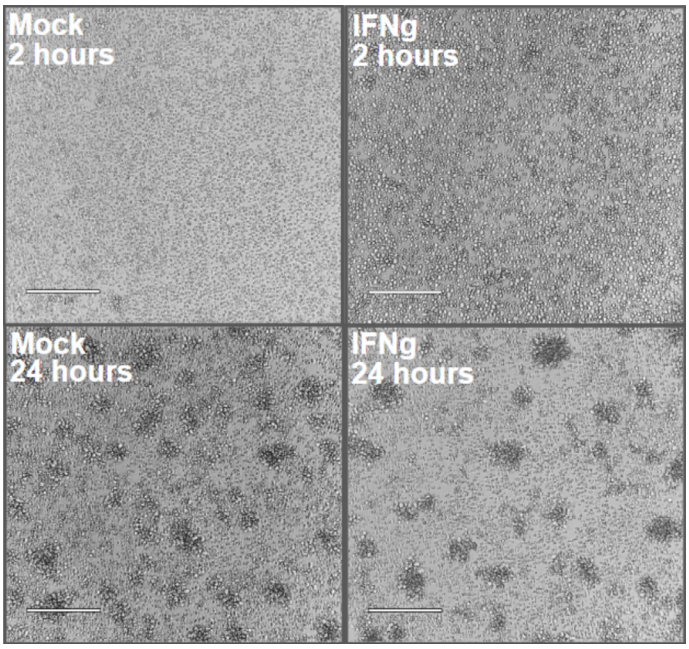


Figure 1: Bright-field images of cultures of edited cells from one donor (Mock transfected and IFNg knockout) at 2 hours and at 24 hours after seeding. Cells were thawed and plated at a density of $1x10^6$ cells/ml in T cell growth medium supplemented with IL2 (see text for details). Cell clumping, which is generally considered an indicator of T cell activation, is more pronounced at 24 hours compared to 2 hours post-seeding, as expected. White scale bar corresponds to 210 um.

Step 3. Genotype

1. EditCo recommends genotyping your Knockout T-cell Pools within 24 hours. To evaluate the genotype of your edited cells you can either use Next Generation Sequencing (NGS) or Sanger sequencing. For single-guide knockouts and knock-in CRISPR edits, if you want to analyze genotypes using NGS, we recommend using CRISPResso. NGS primer sequences are provided in the QC report for your project.

   Alternatively, you can analyze single-guide, multi-guide, and knock-in CRISPR edits using EditCo's Inference of CRISPR Edits (ICE) tool, which relies on Sanger sequencing. Notably, EditCo's ICE tool is currently the only publicly available option for analyzing multi-guide derived CRISPR edits. For Sanger sequencing, PCR primers will be provided upon request. You can reach out to technicalsupport@editco.bio for Sanger primer suggestions for your order. Please note that our Sanger primer recommendations are computed using standard bioinformatic algorithms. They are not validated functionally by EditCo. Instructions on how to isolate genomic DNA, PCR-amplify the targeted region, and prepare for Sanger sequencing are available in our Genotyping protocol. Instructions on assessing knockout or knock-in editing efficiency using ICE are detailed in our ICE Knockout and Knock-in analysis protocols, respectively. For small knock-ins, we recommend identifying the editing genotype of the cells by Sanger sequencing and ICE analysis. For large knock-ins, the inserted sequence can be identified using junction PCR and Sanger sequencing of the PCR product.

Step 4: Limited Expansion of edited T cells (optional)

We tested the ability to expand edited T cells without significant loss of viability for up to 2 weeks, please see guidelines below.

1. After overnight recovery, culture each cell pool in 1 well of a 24-well tissue culture plate at a density of 10^6 cells/ml with the following media:

    a. ImmunoCult™-XF T Cell Expansion Medium supplemented with 50-100 ng/ml of IL2.

    Important: ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator can be added if further expansion is desired. Please be aware that this will result in a transient increase of the PD1 exhaustion marker. PD1 will peak at day 3 and return to baseline levels after 10 days.

    b. Culture plates for 3 days at 37°C and 5% CO2.

2. After 3 days, transfer 1 cell pool to 1 well of a 24-well G-Rex plate in 6 ml XF T Cell Expansion Medium with IL2 (50 - 100 ng/ml).

    Important: 24-well G-Rex plate are critical for successful expansion

3. Replace 80% of the well volume with the complete media XF T Cell Expansion Medium with IL2 (50 - 100 ng/ml) once a week or if medium turns too acidic.

    Note: Please **do not** resuspend the cells.

4. If you wish to freeze your cells, please use the CryoStor® CS10 Freeze Media.

Step 5: Performing Functional Assays

1. Perform functional assays, such as cytokine production or cell killing, as appropriate and ideally within 10-14 days from thawing cells, as these are primary immune human cells with a finite lifespan and prolonged expansion may alter their functional responses.

Additional Information

For an up-to-date list of all protocols and other resources, please visit this link.

For technical assistance, contact our Scientific Support Team at technicalsupport@editco.bio.

For common FAQs, please visit this link.