Engineered Immortalized Cells Quick Start Guide

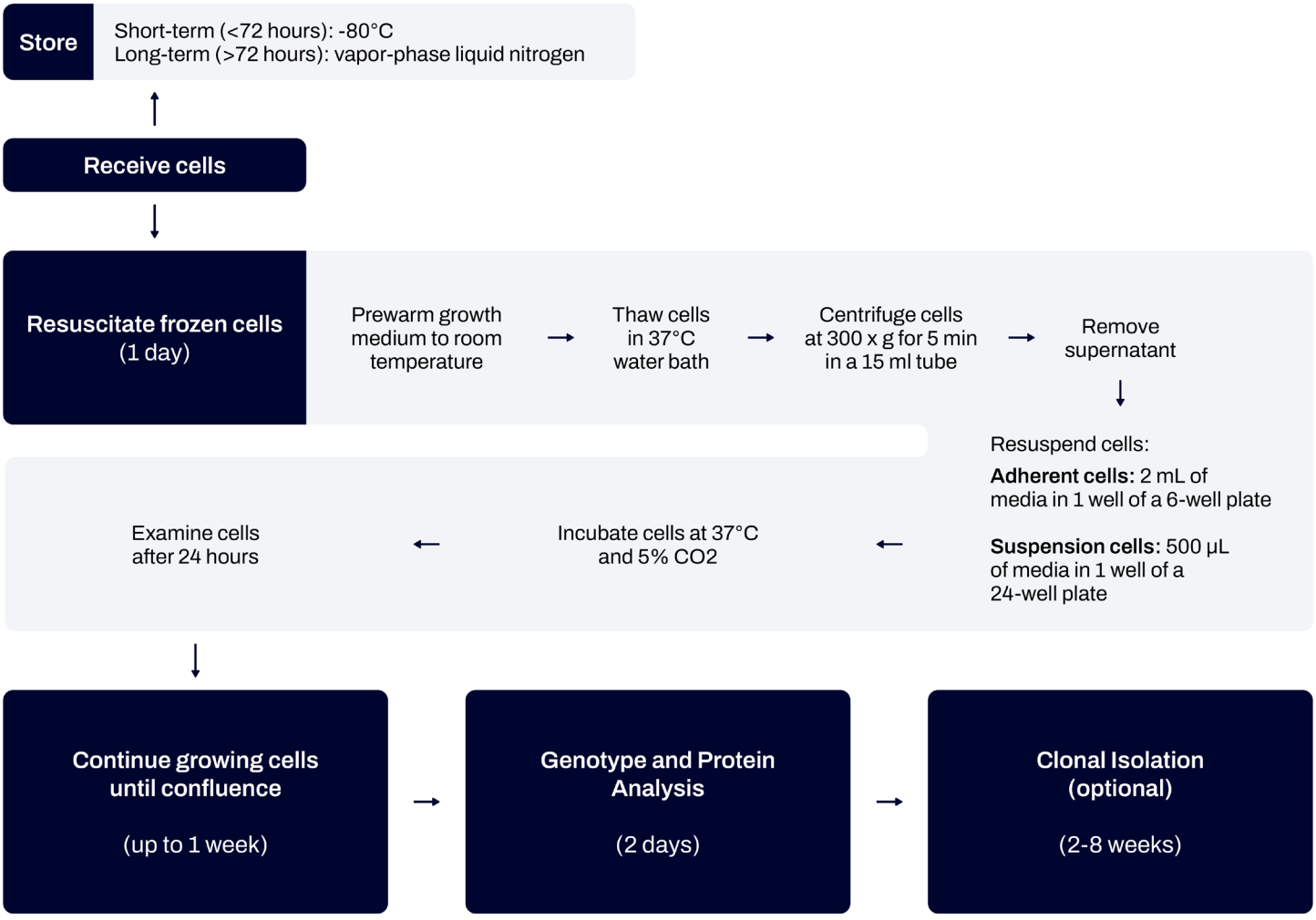
Thank you for choosing EditCo Engineered Immortalized Cells for your CRISPR experiment!

Your cells were created using high-quality chemically modified synthetic sgRNA and SpCas9 transfected as RNPs to ensure high editing efficiencies and without the use of any selection markers that can negatively affect cell biology. Knock-ins are generated using either ssDNA or plasmid, depending on the insert size. This Quick Start Guide can be used for all Immortalized cell pools, clones, knockout and knock-in cells. In this guide, we include important information on how to store, resuscitate, and evaluate these cells. For additional details about your order, please reference the corresponding QC report.

Important Considerations

- **Please note that if the Immortalized 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. Broad-spectrum antibiotics/antimycotics can be added to the medium to prevent unwanted contamination. For more details on how we produce our Engineered cells, please see our Cell Engineering 101 eBook.
- **Cells come frozen in the following freezing media: Recovery™ Cell Culture Freezing Medium from Gibco**. This medium contains 10% DMSO, is a ready-to-use medium, and no additional supplements are needed. To make your own frozen cell stocks, you can either use this, or the ATCC recommended media + % DMSO. Please follow the % DMSO recommended by the ATCC website. If it's not specified, we recommend using 10% DMSO.
- **The recommended growth medium for different cell types is available on the ATCC or other suppliers' websites**. However, please feel free to contact the Scientific Support Team at technicalsupport@editco.bio to confirm the media conditions for your cell line. If you provided the cell line, we used the media conditions you specified.
- For pool populations, if the knockout or knock-in editing causes the cell growth dynamics to shift, the cell population may change over time. For clonal populations, the genotype of your edit is stable and will not change over time.
- Please refer to our QC Report User Guide if you have any questions regarding the information included in your QC report.

Workflow Schematic



Materials Required

Name
Complete cell growth medium (ATCC or other supplier recommended)*
70% Ethanol (in a spray bottle)
Sterile 15 ml centrifuge tubes (Multiple vendors e.g, Thermo Fisher Scientific)
Sterile pipettes (Multiple vendors e.g, Thermo Fisher Scientific)
Tissue culture treated flasks, plates or dishes (Multiple vendors e.g, Thermo Fisher Scientific)
Water bath
Centrifuge
Biosafety Cabinet at appropriate containment level
Cell Incubator

*The recommended growth media for different cell types are available on the ATCC or other suppliers' websites. 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. 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 long-term storage (>72 hours). With appropriate storage, cells can be kept for as long as needed.

   Important: Make a note of cells state upon delivery. They should still be frozen, tip the vial over to make sure the cryopreservation media is still solid. Please contact the Scientific Support Team at technicalsupport@editco.bio in case your cells arrive thawed or if there is any other visible damage.

2. If desired, cells can be thawed immediately after arrival. Please follow step 2 below. We highly recommend confirming the genotype of cells and their protein expression within 1-3 passages (see Step 3 below) and creating your own frozen cell stocks as soon as possible.

Step 2. Resuscitate the frozen cells

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

1. Prewarm growth medium (recommended by ATCC or other supplier) to room temperature. Please contact the Scientific Support Team at technicalsupport@editco.bio if you have questions on what medium to use.

2. Prepare the culture vessel (label with the cell line name, passage number, and date).

3. Remove a tube containing the frozen cells from storage and quickly place in a 37°C water bath. Please leave the top of the tube out of the water, **do not** submerge the entire tube in the water bath.

4. Rapidly thaw the cells over approx 1-2 minutes by gently swirling the tube in a 37°C water bath. Continuously monitor the tube until only small ice crystals remain.

5. Remove the tube from the water bath and spray it with 70% ethanol and wipe clean.

6. In a biosafety hood, pipette all the contents of the tube (1ml) into a 15 ml centrifuge tube and add up to 10 ml of prewarmed complete cell growth medium to it.

7. Centrifuge the cell suspension at 300 x g for 5 minutes (centrifugation speed and duration may vary depending on the cell type, see guidelines on the ATCC or other suppliers' websites).

8. Using a sterile pipette, remove the supernatant without disturbing the cell pellet.

9. Resuspend the cells pipetting up and down gently (**do not** vortex) in their respective complete cell growth medium (including all necessary components such as serum or growth factors) and count cells (if desired). Suspension cells should be thawed at no less than 2 x 10^5 cells/ml. The recommended cell density is available on ATCC / other supplier websites. Please resuspend cells as indicated below:

   - **For adherent cells:** 2 mL of media in 1 well of a 6-well plate
   - **For suspension cells:** 500 µL of media in 1 well of a 24-well plate

   Important: Cell density plays an important role in recovering the cells post-thaw. Please follow these guidelines carefully to ensure cell revival.

10. Transfer the culture vessel to an incubator set at 37°C and 5% CO2 (or recommended culture conditions as per the cell line, see guidelines recommended by ATCC or other suppliers).

11. Examine the cells after 24 hours to ensure they are healthy (e.g., clear borders, absence of contamination). For adherent cells, ensure that cells have adhered to the plate. Please do not change the media for the first 48 hours. Subculture as necessary (best practices recommend not going past 70% confluence). Prepare enough culture vessels for genotyping, protein analysis, and banking, as desired.

    Important: The cells might take longer than expected to recover and start proliferating post-thaw, cells might take up to 1 week to recover. Please ensure that you have followed the recommendations and thawed at least one vial of the edited and the control cells, to discard any viability difference between these. Please contact the Scientific Support Team at technicalsupport@editco.bio if you experience any cell viability issues with your sales order number. It is also recommended to share any viability data and images in case of any issues in successful cell recovery.

Step 3. Genotype & Characterize Protein

1. EditCo recommends genotyping your cells as soon as possible (within 1-3 passages). 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.

2. For Knockout cells, protein abundance should be conducted to confirm the desired Knockout (e.g., western blot, flow cytometry, ELISA, immunostaining) within the first 7 days after thawing the cells. See our Tips and Tricks: Protein Analysis of Knockouts for an overview of different protein analysis techniques.

Step 4: Isolate Clones (optional)

1. 1. Clonal cell lines can be produced from a cell pool. Please follow the Limiting Dilution & Clonal Expansion protocol to isolate single cells via limiting dilution and generating monoclonal populations.

2. After generating clonal populations, we recommend identifying the editing genotype of the cell clone by Sanger sequencing and ICE analysis and validating protein depletion to confirm the desired knockout. For small knock-ins, we recommend identifying the editing genotype of the cell clone 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. Appropriate downstream assays can be performed.

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.