



Engineered Cell Libraries Quick Start Guide

Knockout Immortalized Cells

Thank you for choosing EditCo's Engineered Cell Libraries (ECL) for your experiment!

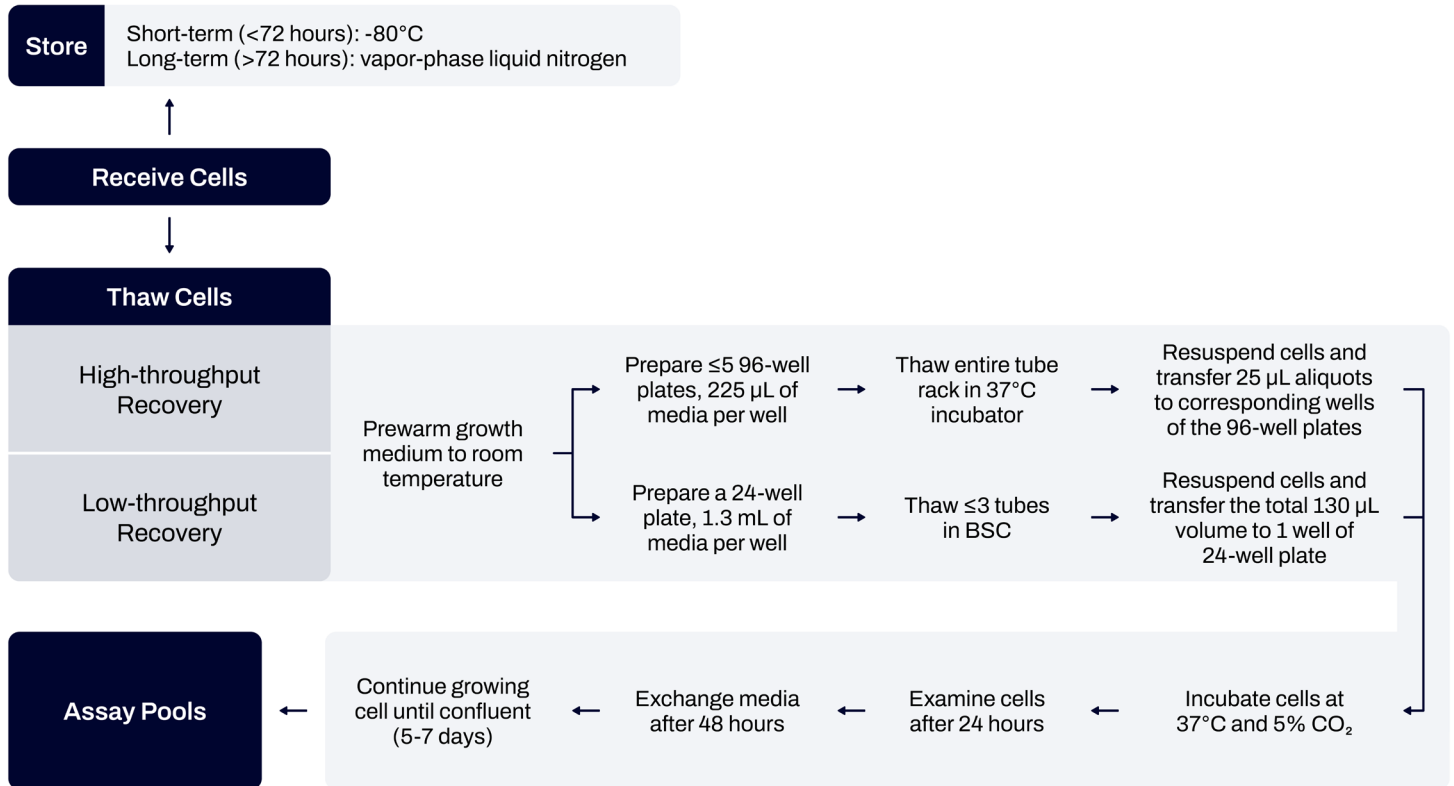
Your library of knockout cell pools was created using high-quality synthetic sgRNAs (designed using a proprietary multi-guide technology) and SpCas9 transfected as RNPs to ensure high editing efficiencies. No selection markers were used to prevent any negative effects on cell biology. In this guide, we include important information on how to store, resuscitate, and culture these pools. For additional details about your order, please reference the corresponding Engineered Cell Library 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.** Seeding cell density plays a key role in recovering the cells post-thaw. Please carefully follow the resuspension and plating instructions included in Step 2.
- Cells are provided in a freezing medium consisting of DMEM/F12, KnockOut™ Serum Replacement, and 10% DMSO.
- Optimal recovery conditions for ECL pools may vary depending on the biology of the parental cell line. For this reason and due to the novelty of the ECL product format, first-time users are encouraged to confirm the successful recovery of cryopreserved cells using one of the provided control pools before conducting experiments.
- Editing of some gene targets may negatively impact cell growth dynamics, causing frequencies of edited cells in ECL pools to decrease over time in culture. For this reason, it is recommended that functional assays are performed within 3 passages of culturing ECL pools.
- **The recommended growth media for different cell types is available on the ATCC or other suppliers' websites.** Broad-spectrum antibiotics/antimycotics such as 1% Penicillin-Streptomycin or 1% Antibiotic-Antimycotic can be added to the medium to prevent unwanted contamination. However, please feel free to contact [EditCo's Technical Support](#) to confirm the media conditions for your cell line. If you provided the cell line with recommendations for culturing, we used the media conditions you specified.
- If you wish to make your own frozen cell stocks, you can use the ATCC recommended media with DMSO added. Please follow the % of DMSO recommended by the ATCC website. If it's not specified, we recommend using 10% DMSO.



Workflow Schematic





Product Format

Engineered Cell Libraries consist of edited cell pools provided in individual 0.5 mL tubes arrayed in a 96-tube format rack. This format allows for the application of individual pools or subsets of pools in downstream assays depending on your experimental needs and capacity constraints. Each tube contains ~130 μ L of cell suspension. The cell freezing medium used for cryopreservation of the cells contains 10% DMSO. For your reference, the tube manufacturer and part numbers are provided below.

- **Individual Tube:** 0.5 mL tube (Azenta 68-0703-11).
- **Tube Rack:** The tubes are in a 96-format rack (Azenta 66-51004) and secured with a removable and lockable lid.
- **Tube Label:** Each tube has 2D code on its base, 1D linear barcode and human-readable number on its side.
- **Tube Seal:** Your tubes are sealed according to your preference (selected before the project is booked).
 - **Option 1 - Mineral Oil:** The tubes are sealed with a 100 μ L frozen layer of sterile-filtered mineral oil (~230 μ L total volume). During thawing of cells, the mineral oil will also thaw to “unseal” the tube, allowing pipetting access to the cells. **Please be careful to avoid inverting tubes once they are thawed. Failure to do so may result in loss of cells.**
 - **Option 2 - Caps:** The tubes are capped with external threaded caps (Azenta 68-53111-10N). The tubes can be individually uncapped/capped by screwing off/on the cap. Alternatively, the full rack of 96 capped-tubes can be uncapped simultaneously with the [IntelliXcap Automated Screw Cap Decapper/Recapper instrument](#) from Azenta fitted with a 96-format cartridge (Azenta 48-8013-02).



Materials Required

Name
Cell growth medium (ATCC or another supplier recommended) *
70% isopropyl alcohol
Kimwipes
Sterile 24-well or 96-well tissue culture treated plate(s)
Sterile micropipette tips
Micropipettes
Microscope for monitoring cell recovery and confluency
Biosafety cabinet at appropriate containment level
37°C and 5% CO ₂ cell incubator
Tongs suitable for manipulation of individual tubes in a 96-format rack.

*The recommended growth media for different cell types are available on the [ATCC](#) or other suppliers' websites. Please contact EditCo's technical support team if you have questions on which medium to use.

Optional Material

- [IntelliXcap Automated Screw Cap Decapper/Recapper instrument](#) with Cartridge for 96 format external threaded caps (Azenta 48-8013-02)



Step 1. Receive Cells & Place in Storage

1. EditCo's ECLs ship frozen on dry ice. After receiving cells, immediately place them in storage. Frozen cells should be stored at -80°C for short-term storage (≤ 72 hours) or in vapor-phase liquid nitrogen for longer term storage (> 72 hours). ECL tube racks should be placed into standard freezer boxes prior to storage in liquid nitrogen racks. When stored properly, ECL pools can be kept up to 2 years based on test data.

Important: Storage of ECL racks in **liquid-phase** liquid nitrogen is **not** recommended. Liquid nitrogen in the liquid phase could accumulate inside the tubes, raising contamination and safety risks.

2. If desired, cells can be thawed after receipt. Please follow the thawing instructions in Step 2.

Step 2. Resuscitate the Frozen Cells

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

Cells can be successfully recovered using either of the following methods:

High-throughput Recovery (Processing Batches of Tubes into 96-well Culture Plates)

1. Pre-warm growth medium (recommended by ATCC or another supplier) to room temperature. Please contact EditCo's [Technical Support](#) if you have questions on what medium to use.
2. Prepare up to five 96-well tissue culture plates with 225 μL of growth medium per well, corresponding to the pool layout of the tube rack.
3. Remove the tube rack from cold storage and wipe the exterior with a Kimwipe and 70% isopropyl alcohol to remove any extra ice and moisture.
4. Place the tube rack in a 37°C incubator to thaw. Refer to the following table for average thaw durations. The tubes in the center of the rack will take longer to thaw than the tubes in the outer positions of the rack. **DO NOT INVERT TUBES.**

Note: If tubes are sealed with mineral oil, the mineral oil will thaw first.

Tube Position	Time to thaw with passive thawing in 37°C incubator
D1	25 minutes
D2	30 minutes
D3	34 minutes
D4 - D6	40 minutes

5. After thawing, wipe the exterior of the tube rack with 70% isopropyl alcohol and place it into a biosafety cabinet. Remove the rack lid and use tongs to lift the central tubes and visually confirm the tube is completely thawed.
 - a. If the tubes are not completely thawed, reinsert the tubes and reattach the rack lid. Place the tube rack back in the incubator for an additional 5 minutes. Repeat step 5 to confirm complete thawing.



6. Set the corresponding multi-channel micropipette to 25 μ L and gently resuspend the cells by pipetting up/down 3-5 times.

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

- a. If sealed with mineral oil (seal option 1): depress the pipette plunger and insert the tips through the mineral oil to a position slightly above the bottom of the tubes.
 - b. If sealed with external threaded caps (seal option 2): unscrew the caps manually or with the IntellXcap automated instrument, depress the pipette plunger and place the tips slightly above the bottom of the tubes.
7. Aspirate a 25 μ L aliquot of cells from the bottom of the tubes and dispense into the corresponding wells of a prepared 96-well plate.
 - a. Repeat step 7 with the same tubes and tips, aliquoting cells into corresponding wells of the additional 96-well plates prepared in step 2.
 8. Exchange for new pipette tips and repeat steps 6-7 for all thawed tubes of the tube rack containing cells.
 9. Check the recipient wells of the 96-well plates for deposited cells under a microscope.
 10. Store the 96-well tissue culture plates in a 37°C and 5% CO₂ incubator.
 11. After 48 hours, exchange medium with fresh growth medium.
 12. Monitor confluence. Cells typically reach confluence in 5–7 days.

Important: The cells might take longer than expected to recover and start proliferating post-thaw. This timeframe will be determined in part by the growth properties of the parental cell line and any fitness consequences associated with specific gene knockouts.

Low-throughput Recovery (Processing Individual Tubes into a 24-well Culture Plate)

1. Pre-warm complete growth medium (recommended by ATCC or another supplier) to room temperature. Please contact EditCo's [Technical Support](#) if you have questions on what medium to use.
2. Prepare a 24-well tissue culture plate with 1.3 mL of growth medium per well.
3. Identify tube(s) of interest and their position(s) in the rack before removing the rack from cold storage.
4. Prepare a container with an adequate amount of dry ice for storage during the thaw process.
5. Transfer the tube rack from cold storage to the dry ice container and transport to a biosafety cabinet.
6. Wipe the exterior of the tube rack with 70% isopropyl alcohol before placing the rack in a biosafety cabinet.
7. Using tongs, remove up to three tubes at a time from the tube rack after removing the rack lid. Place the tubes in a tube holder to prevent tipping.



8. Immediately reattach the rack lid and return the tube rack to the dry ice container.
 - a. Return the tube rack to long-term or short-term cold storage if no other tubes require thawing.
9. Gently roll the tubes between fingers until completely thawed (1–2 minutes). **DO NOT INVERT TUBES.**

Note: If sealed with mineral oil, the mineral oil will thaw first.

10. Set the corresponding single-channel micropipette to 130 μ L and gently resuspend the cells by pipetting up/down 3–5 times.

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

- a. If sealed with mineral oil (seal option 1): depress the pipette plunger and insert the tip through the mineral oil to a position slightly above the bottom of the tube for mixing.
 - b. If sealed with external threaded caps (seal option 2): manually unscrew the caps, depress the pipette plunger and place the tip slightly above the bottom of the tube for mixing.
11. Aspirate the total 130 μ L volume of cells from the bottom of the tube and dispense into the prepared 24-well plate.

Note: If sealed with mineral oil, transfer of a small amount of mineral oil to the well is normal. The mineral oil will be removed the next time you perform media exchange.
12. Repeat steps 6–11 until all tubes of interest have been thawed and transferred into the 24-well plate.
13. Return the frozen tube rack containing unused cell tubes to long-term or short-term cold storage.
14. Check the recipient wells of the 24-well plate for deposited cells under a microscope.
15. Store the 24-well tissue culture plate in a 37°C and 5% CO₂ incubator.
16. After 48 hours, exchange medium with fresh growth medium.
17. Monitor confluence. Cells typically reach confluence in 5–7 days.

Important: The cells might take longer than expected to recover and start proliferating post-thaw. This timeframe will be determined in part by the growth properties of the parental cell line and any fitness consequences associated with specific gene knockouts.



Step 3. Assay or Expansion of Cell Library

1. EditCo recommends assaying your cells as soon as possible. The genotype of your edited cells may drift as cells are passaged.
2. Genotyping is not required for ECL pools. However, if you wish to genotype pools, instructions for isolating genomic DNA, PCR amplification, and preparing samples for Sanger sequencing can be found in our [Genotyping protocol](#).
 - a. To evaluate the genotype of your edited cells you can use Sanger sequencing and analyze sequences using EditCo's [Interference of CRISPR Edits \(ICE\) tool](#). Instructions on assessing knockout editing efficiency using ICE are detailed in our ICE [Knockout](#) analysis protocol.
 - b. The PCR primer sequences used in Sanger sequencing are included in the Design Information section of the supplementary data.

Summary of Thawing and Media Guidelines for Recovery of Engineered Cell Libraries

Thaw Method	High-throughput Recovery	Low-throughput Recovery
Thaw Batch Size	96 tubes	≤3 tubes (recommended)
Thaw Condition	37°C Incubator	Roll between fingers
Thaw Time	25-40 minutes	1-2 minutes

Plating Method	96-well Tissue Culture Plate	24-well Tissue Culture Plate
Volume of Growth Medium per Well	225 µL	1.3 mL
Volume of Thawed Cells per Well	25 µL	130 µL
Number of Replicates per Tube	≤5	1

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](#).