# Cell Banking Kit

*Quick start guide*

The following procedure is a starting point for cell banking. This design also accommodates filling via syringes, gravity or by pressurized vessel. Contact Entegris for technical support for alternative filling and freezing methods.

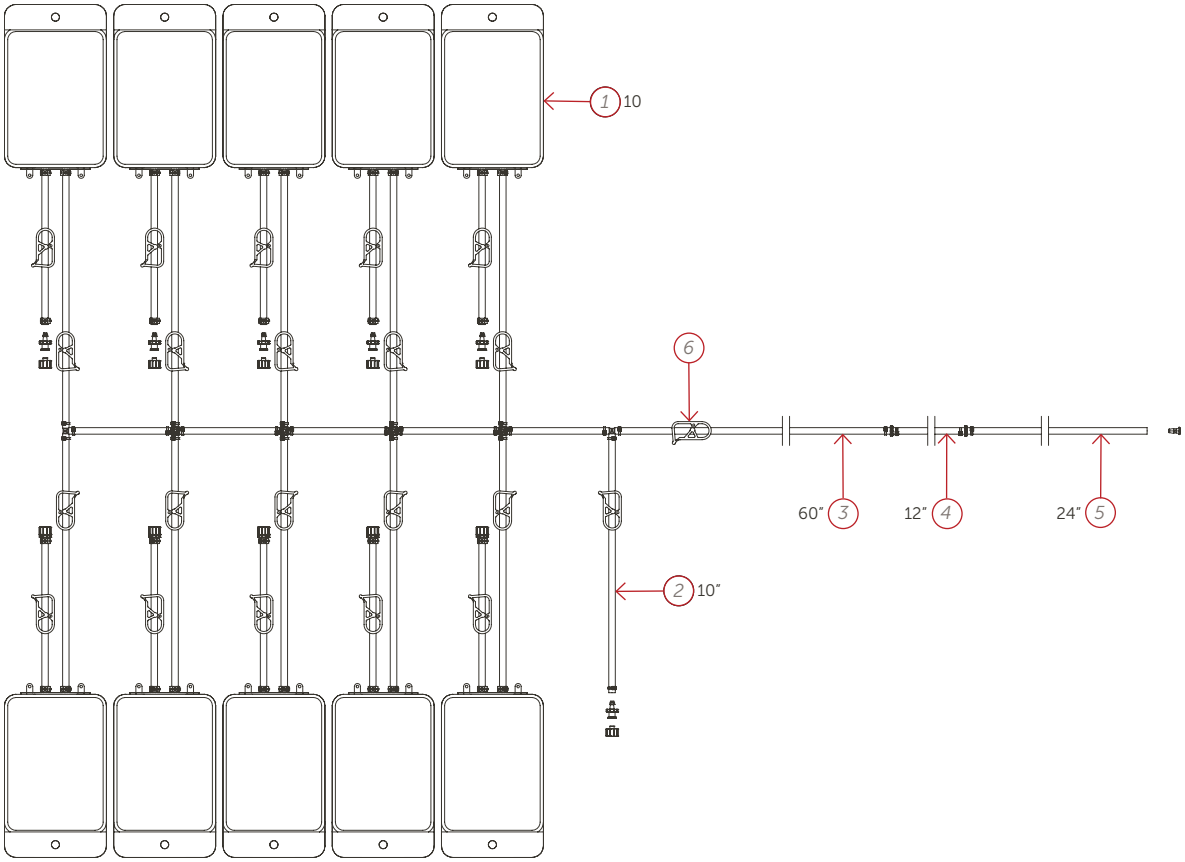
## WHAT'S IN THE BOX?

| Part number | Description | What's included |
|---|---|---|
| SU-ADM-000048K | Aramus™ Cell Bank Manifold Kit, 5× 100 mL | Five 5-bag manifolds, 5×4 EVA overwraps |
| SU-ADM-000049K | Aramus Cell Bank Manifold Kit, 10× 100 mL | Five 10-bag manifolds, 10×4 EVA overwraps |
| SU-ADM-000050K | Aramus Cell Bank Manifold Kit, 5× 250 mL | Five 5-bag manifolds, 5×4 EVA overwraps |
| SU-ADM-000051K | Aramus Cell Bank Manifold Kit, 10× 250 mL | Five 10-bag manifolds, 10×4 EVA overwraps |

### ADDITIONAL ACCESSORIES (IF ORDERED)

Ask your account manager for additional information on ordering:

- Cassettes
- Additional sterile overwraps




**NOTE:** To most effectively use this system, Entegris recommends that you have these pieces of equipment and materials available. Please reach out to your account manager for recommendations best suited for your application:

- Balance for weighing bags (recommended)
- Tube welder
- Peristaltic pump
- Cryo assembly for tare weight

## GETTING STARTED

1. Locate manifold near to workspace and bioreactor prepared for harvest.
2. Remove manifold from the double bag; leave overwraps inside bag until ready to use.
3. Clamp-off all lines.
4. Weld the cell inlet line (5) to the source line and ensure good weld.
5. Place peristaltic pump at transfer tube segment (4).
    a. Note: Assembly maximum pressure is (10 psi); suggested flow rate 280 mL/min (e.g. 350 RPM on Masterflex Easyload II head with included #16 tubing – ⅛" ID x ¼" OD). Maximum flow rate and pressure may be lower due to cell-line specific requirements.

6. If applicable, connect cryoprotectant to cryoprotectant inlet line (2); alternately add appropriate amount of cryoprotectant to bioreactor or pooling bag as per internal procedure.
7. Open the fill line clamps to the bag farthest from the pump.
8. Place the bag to be filled on a balance and zero it.
9. If applicable, administer cryoprotectant to first bag – if not already added to bioreactor. You may do this step for all bags at this time or sequentially depending on cell line tolerance for contact time with DMSO.

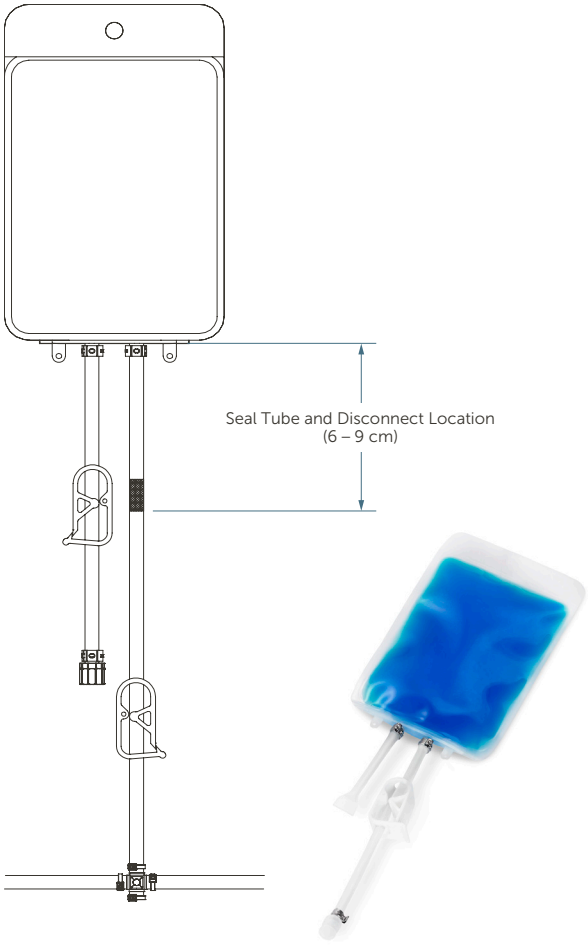
   **NOTE:** Aramus bag integrity is not significantly affected by exposure of 100% DMSO up to 21 days.

10. Fill cells into bag until the appropriate amount is in the bag. Stop the pump. Clamp off line to the filled bag.

    a. If you will be freezing in Entegris provided cassettes, do not exceed the following:

| Cassette part number | Aramus bag size | Aramus fill volume (in cassettes) |
|---|---|---|
| SU-FS-0.10-C2 | 100 ml | 100 ml |
| SU-FS-0.25-C2 | 250 ml | 200 ml |

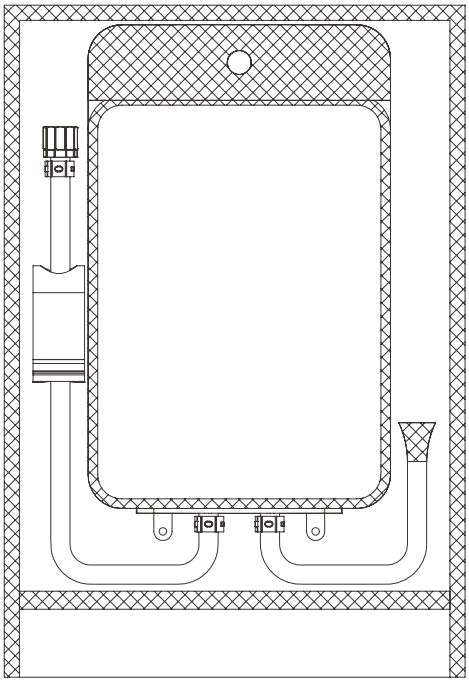
11. Proceed to filling subsequent bags. Open their respective clamps, one at a time, repeating the above steps until all bags are filled.
12. Use tube sealer to seal and disconnect tubing from manifold. Bags can be disconnected after filling each bag or can be disconnected after filling all bags. Entegris recommends sealing tube length to 8 cm.




13. Samples should be taken at appropriate times according to SOP and may vary by manifold design if using a custom system.

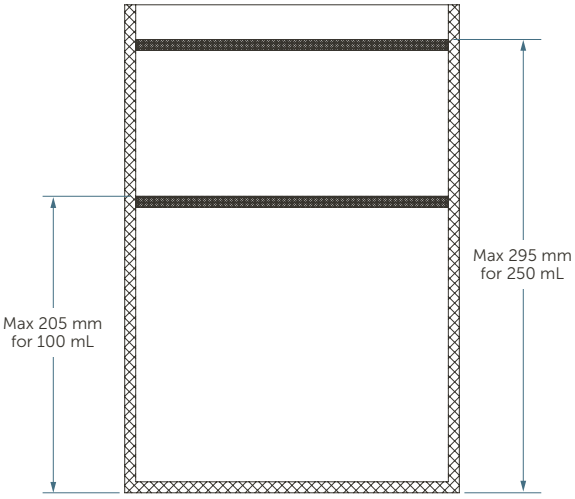
## PREPARING FOR FREEZING

1. If bags need to be weighed and labeled, they should be done at this time. Use Zebra Cryocool labels or equivalent labeling to resist -196°C (-321°F). Labels should not be placed on fluid contact locations of the bag and instead should be placed on top near hanging feature.
2. Remove overwrap(s) from bag and place Aramus bag inside.
   a. Insert Aramus bag chamber side first into overwrap pouch and guide each tube towards each side of the bag as pictured below.

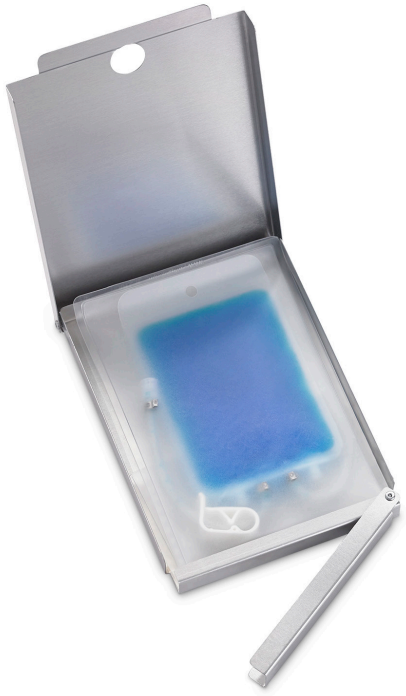


3. Overwrap should be sealed according to the following:

| | |
|---|---|
| 100 mL pouch size | Max Length: 205 mm |
| 250 mL pouch size | Max Length: 295 mm |
| Recommended sealer | Packworld PW3016 and PW3024 or similar |
| Sealer settings | 130°C (266°F), 50 psi, 5 seconds |



4. After bag is enclosed in overwrap, additional label(s) can be applied if necessary. Fold excess overwrap material and place bag into cassette. Bag should be inserted tubing side first into cassette groove to ensure proper fit. Close cassette and apply label if not yet completed.



5. Place cassettes directly into cryorack or -80°C (-112°F) controlled rate freezer (recommended). Otherwise, passive freezing can be achieved in a -80°C (-112°F) laboratory freezer.
6. Once bags reach -80°C (-112°F) they can be transferred into long term storage, LN2 freezer or dewar either above the surface of the liquid or fully submerged until they are ready to be used.
7. If cells will be shipped to another site, prepare cassettes/racks for shipment. Consult Entegris for recommendations on shipping and transferring cells.

## PREPARING FOR USE OF CELL BANK

1. Prepare bioreactor media and bioreactor as per internal procedures. Depending on the cell density and cell type, a single 100 – 250 mL bag can feed a 25 – 125 L bioreactor.
2. Thaw in a water bath (within overwrap) at 37°C (99°F), room temperature, or 4°C (39°F) depending on cell type.
   a. Conduct manual agitation of the cells periodically to ensure even thawing.
   b. It is recommended to completely dry the overwrap (or bag assembly) before making any connections, spray or wipe down with sterile IPA. Bag can be placed in an isolator if the environment of the room is considered high risk for connection sterility.
3. Conduct cell wash, media exchange, or dilution to prevent cell death from cryoprotectant. Entegris mesh bag* (provided separately) can be utilized, or cells may be stored in the Aramus bag in an incubator until acclimatized.
   a. The Entegris mesh bag can be utilized to create a completely sterile microcarrier or cell separation process using a streamlined bag system. Equipped with a peristaltic pump, chemically inert PET mesh and ¼" standard port size, each bag can be customizable to adapt to existing processes and is functional with a vast majority of bead sizes.
4. Samples may be taken at any point in the process.
5. Connect cell containing bag to bioreactor, transfer to the bioreactor via gravity, peristaltic pump or head pressure as appropriate.
   a. For best recovery, cells should be agitated while transfer is occurring. The cell banking bag may be washed with sterile media for complete recovery of cells into bioreactor.
6. Aseptically disconnect cell bank bag (if required) and follow procedure for bioreactor use.

## LIMITED WARRANTY

Entegris' products are subject to the Entegris, Inc. General Limited Warranty. To view and print this information, visit entegris.com and select the Legal & Trademark Notices link in the footer. Entegris does not warrant any failure in the case of customers using unapproved foreign components.

## FOR MORE INFORMATION

Please call your Regional Customer Service Center today to learn what Entegris can do for you.
Visit entegris.com and select the Contact Us link to find the customer service center nearest you.

## TERMS AND CONDITIONS OF SALE

All purchases are subject to Entegris' Terms and Conditions of Sale. To view and print this information, visit entegris.com and select the Terms & Conditions link in the footer.

Entegris

Corporate Headquarters
129 Concord Road
Billerica, MA 01821
USA

Customer Service
Tel +1 952 556 4181
Fax +1 952 556 8022
Toll Free 800 394 4083