

FAQ about Custom Primary Endothelial Cell Isolation**Question 1:** *What method is used for the isolation of primary endothelial cells?*

The method we use to isolate primary endothelial cells was developed based on a combination of established and our proprietary methods. These cells are isolated with anti-PECAM-1 antibody-conjugated magnetic beads.

Question 2: *What passage number would they be?*

You will receive endothelial cells from passages 2 or 3, depending on cell purity.

Question 3: *How many passages can primary cells be maintained?*

Primary endothelial cells can be expanded 3-6 passages by the split ratio of 1:2 under the cell culture conditions specified by Cell Biologics (Recommended Mouse Endothelial Cell Culture Medium: M1168 or M1168CO).

Question 4: *How many cells should you expect to receive from one order?*

- 1) There are 1-2 million cells/frozen vial provided with each order depending on cell types; 2-4 million cells can be made as per your request.
- 2) The cells are frozen at one million cells/ml per vial or per request you can receive proliferating cells in a T25 flask/ 0.6-1 million cells or a T75 flask/2.5-3 million cells.
- 3) Cell Biologics ships frozen cells on dry ice to anywhere in the world. On receipt, frozen cells can be transferred to liquid nitrogen until needed experimental use. Live cell shipment is also available on request.

Question 5: *How long does it take for a custom cell culture?*

Delivery time is usually 3-5 weeks from the time we receive your live mice or tissue samples.

Question 6: *How do we test our cells?*

We test primary endothelial cells for expression of markers using antibodies, VE-cadherin or PECAM-1 (CD31) by fluorescence staining as described on Cell Biologics website. Also, you will receive cell morphology micrographs gratis and cell fluorescent antibody image will be provided for a modest fee on request.

Question 7: *How is cell purity estimated?*

Primary endothelial cells produced from Cell Biologics display typical spindle morphology/or cobblestone morphology under light microscopy and show VE-cadherin (CD144) or CD31 staining at cell-cell junction as demonstrated by fluorescence staining.

Question 8: *How can the knockout or transgenic cells be successfully isolated?*

on request, Cell Biologics' cell strains are only made available to researchers after meeting our standards. Because it is not possible to fully characterize or capture each cell strain in the cell strain data sheets, we cannot guarantee each cell strain's phenotype will meet all expectations after cell isolation and in vitro cell culture. To ensure that Cell Biologics' cells will meet the needs of individual research projects or when requesting a cell strain that is new to your research, we suggest ordering and performing tests on a small number of cells (1-2 vials) to determine suitability for your particular project.

Question 9: *What laboratory applications can these cells be used for?*

Primary endothelial cells can be used for a variety of purposes (e.g., assays of cell-cell adhesion, migration, vascular tube formation, angiogenesis assays and many other applications) Standard biochemical procedures can be performed using endothelial cell cultures include RT-PCR, Western blotting, immunoprecipitation, or immunofluorescent staining or flow cytometry, et al.

Question 10: *How much does isolation of endothelial cells cost?*

- 1) \$1200 for 1-2 million cells/frozen vial per custom order of one cell type.
- 2) \$1200 for one T25 flask (0.5-0.8 million cells).
- 3) \$1300 for one T75 flask (2.5-3 million cells).

Discount pricing is offered when orders are for more than three types of cells derived from different organs or tissues.

Question 11: *What types of samples do we accept for cell culture for custom orders?*

- 1) You can send animal organs (lungs, heart, livers, brain, kidney, dermal etc) from 5-8 mice. The organs or tissues collected from younger animals (1-5 week) provide the better results. If you choose this approach, we will provide you with the specific protocol for collecting and sending us tissue.
- 2) You also can deliver your live mice (1-6 weeks) directly to us. Younger the mice (1-2 weeks) provide better results and typically each special order requires 6-10 mice. You can ask your animal facility or company to contact us for animal delivery.

Question 12: *Would one tissue type have to be specified, or can endothelial cells from multiple locations be isolated?*

Cells derived from one or multiple types of tissues or organs can be provided from each mouse strain on request.

Question 13: *Will Cell Biologics provide the isolation methods?*

We will provide you with a brief synopsis of our cell isolation method for the purpose of your publication. All Cell Biologics methods are proprietary.

Question 14: *What kind of support is provided for long term projects?*

For long term use of our custom orders, one of our representatives will work with you on any special requests your project may entail, along with providing special discount pricing, upon approval of written contract.

Question 15: *How do we inquire more about a custom order?*

You can either send an email to service@cellbiologics.com with the details of your request including the type of cells needed and quantity of cells and one of our representatives will contact you for further information.

Authorized Uses of Cell Biologics' Products

Primary endothelial cells from Cell Biologics are distributed for laboratory research purposes only. Our products are not authorized for human use. Transfer or resale of any Cell Biologics' cells or products from the purchaser to other markets, organizations, or individuals is prohibited by Cell Biologics without the express written consent of the company. Cell Biologics' Terms and Conditions must be accepted before submitting an order.

For a detailed list of the cells available from Cell Biologics, please go to our website at <http://www.cellbiologics.com>. Custom Primary Cell Isolation Service is available internationally.

If you would like to use of Custom Cell Isolation Service, please contact us at service@cellbiologics.com. Thank you for your interest in Cell Biologics' Cell Isolation Service and we look forward to working with you in the near future.

Cell Biologics