Aortic Mouse Endothelial Cells

Catalog Number	M1010
Product Name	Aortic Mouse endothelial cells
Storage	37°C $C0_2$ incubator
Product Format	Proliferating culture
Cells Number	>90% confluent in T25 flask

***Caution:** Although primary cells are tested pathogen-free, investigators should handle these cells with caution and treat all animal cells as potential pathogens, since no test procedure can completely guarantee the absence of infectious agents. Proper precautions must be taken to avoid exposure. Always wear proper protective equipment (Gloves, safety glasses, etc.) when handling these materials. We recommend following the universal procedures for handling products of animal origin as the minimum precaution against contamination.

GENERAL INFORMATION

Aortic mouse endothelial cells were isolated from aorta of C57BL6 mice. The cells are shipped in proliferating culture or frozen vial with a confluence of > 90% (the cells are provided at passage 2). ENDO-Growth medium (EGM-2102) containing 5% serum and growth supplement is recommended for cell culture and these cells have an average minimum population doubling levels 6-8 when cultured following the detailed protocol. Cells are tested negative for common experimental animal pathogens and mycoplasma in vitro. When you receive the cells, leave the flask in 37°C C02 incubator for 1 hour first and then replace the transport medium with fresh ENDO-Growth medium (EGM-2102). Let the cells grow for 24 hours before subculture.

CELL CHARACTERIZATION

PECAM1	>95% positive by immunofluorescence
VE-Cadherin	>95% positive by immunofluorescence
Aortic Mouse endothelial cells	Negative for mycoplasma

PRODUCT USE AND SHIPPING STATUS

Product Use	Aortic Mouse endothelial cells are for research only
Shipping Status	Proliferating culture in T25 flask

Alphabioregen Inc. 607 Boylston street Suite 384L Boston, MA 02116 USA.
sales@alphabioregen.com Fax: (617) 844 - 6082. Tel: (888) 982 - 8905

T25 flask

*Coating T25 flasks. Add 2 ml AlphaBioCoat (AC001) into 3- T25 flasks and ensure entire interior surface is coated with the solution. After 30 minutes, dispose of AlphaBioCoat (AC001) by aspiration. Gently rinse and aspirate the flask with Phosphate Buffer Solution (1XPBS-001). The flask is now ready for use (no need for overnight incubation when coated with AC001). Add fresh media to flask, if color changes from pink to yellow, discard the media, and add fresh media to each flask.

1. Inspect to make sure Flask is at 90% confluence, if not remove transport media, and add 5ml of fresh media to the flask. Place flask in 37°C incubator until cells are at 90% confluence. Change media every 2 days.
2. If flask is at 90% confluence, aspirate transport media from flask.
3. Rinse T25 flask containing cells with 5 ml 1XPBS (1XPBS-001).
4. Gently aspirate out the PBS after rinsing, and discard.
5. Add 2ml of RT trypsin/ EDTA to T25 flask containing cells (ensure entire interior surface is covered.
6. Place T25 flask containing cells into 37°C incubator for 1 or 2 minutes (cells will normally come off of the surface within 1 or 2 minutes).
7. Suspend the cells with 15ml of ENDO-Growth medium (EGM-2102) and transfer equally into 3 pre-coated T25 flasks (the cells are now at a subculture ratio of 1:3.
8. There is no need to spin cells during subculture.
9. Proliferating cell culture: ENDO-Growth medium (EGM-2102) should be changed every 2 days. The cells normally become confluent within 7 days (when split at a 1:3 ratio)
10. Use ENDO- Basal media (EBM-002) containing 0.5% FBS to induce quiescent cells (after 18-24 hours).