

**C57BL/6 Mouse Bone Marrow Macrophages- Frozen Cells**Catalog No. **C57-6030F**

Suggested Medium: Macrophage Medium /w Kit (500 ml)

Catalog No. **M3368****Product Description**

C57BL/6 Mouse Bone Marrow Macrophages are derived from the tibias and femurs of pathogen-free laboratory adult mice. C57BL/6 Mouse Bone Marrow Macrophages are grown in 100 mm treated tissue culture dish and incubated in *Cell Biologics'* Culture Complete Growth Medium for 5-7 days. Cultures are then expanded. Prior to shipping, cells are detached from flasks and immediately cryo-preserved in vials. Each vial contains at least  $6 \times 10^6$  cells and is delivered frozen. C57BL/6 Mouse Bone Marrow Macrophages are negative for bacteria, yeast, fungi, and mycoplasma. C57BL/6 Mouse Bone Marrow Macrophages are tested for expression of markers using antibodies, CD11b by flow cytometry. They can be expanded on a multiwell culture plate ready for experiments under the cell culture conditions specified by *Cell Biologics*.

**Laboratory Applications**

C57BL/6 Mouse Bone Marrow Macrophages can be used in standard biochemical procedures include PCR, Western blotting, immunoprecipitation, ROS, or cell derivatives for desired research applications.

**Storage of *Cell Biologics* Products**

*Cell Biologics* will ship frozen cells on dry ice. On receipt, immediately transfer frozen cells to liquid nitrogen until ready for experimental use. Live-cell shipment is also available on request.

Never can primary cells be kept at  $-20^{\circ}\text{C}$ .

**Authorized Uses of *Cell Biologics* Products**

C57BL/6 Mouse Bone Marrow Macrophages from *Cell Biologics* are distributed for research purposes only. Our products are not authorized for human use, for in vitro diagnostic procedures, or for therapeutic procedures. Transfer or resale of any *Cell Biologics'* Cells or Products from the purchaser to other markets, organizations, or individuals is prohibited by Cell Biologics. *Cell Biologics'* Terms and Conditions must be accepted before submitting an order.

**Disclaimer**

Although C57BL/6 Mouse Bone Marrow Macrophages are isolated from laboratory mice testing pathogen-free, investigators should handle the cells that they receive from *Cell Biologics* with caution and treat all animal cells as potential pathogens, since no test procedure can completely guarantee the absence of infectious agents.

**Warranty and Liability**

*Cell Biologics'* guarantee applies only to your purchase of *Cell Biologics'* cells with *Cell Biologics'* Media and Coating Solution for appropriate cell culture and cell testing following *Cell Biologics'* online protocols within 35 days from the date of product delivery.

## Primary Cell Culture Protocol

All cell culture procedures must be conducted in a bio-safety cabinet.

Any and all media, supplements, and reagents must be sterilized by filtration through a 0.2 µm filter.

Use aseptic technique to prevent microbial contamination.

Cryo-preserved cells must be stored in liquid nitrogen or seeded immediately upon arrival.

### Medium:

Review the information provided on the *Cell Biologics* website about appropriate culture media (e.g. serum and other supplements). Use pre-warmed (37°C) cell culture media (30-50 ML) to recover cryo-preserved cells and when changing media or splitting cells.

### Coating of flasks or dishes:

Coat sterile culture dishes or flasks with Gelatin-Based Coating Solution (*Cell Biologics*, Catalog No. 6950) for 2 min and then aspirate the excess solution before seeding cells.

### Handling of Arriving Live Cells

When you receive the live cells in a T25 or T75 flask, remove the sticker from the filter cap, and keep the flask with 6-20 ml existing medium in 37°C CO<sub>2</sub> incubator for 1 hour before replacing the desired *Cell Biologics'* cell culture medium. Either split the 95-100% confluent cells from a T25 flask to a T75 flask after 1 hour or let the cells grow in the T25 flask with the desired Medium (such as M3368) for 12 hours before subculturing cells. The recommended split ratio for primary cells is 1:2.

### Cell recovery from cryovial:

- Quickly thaw cells in cryo-vial by incubating them in a 37°C water bath for <1 min until there is just a small bit of ice left in the vial.
- Promptly remove the vial and wipe it down with 70% ethanol.
- Transfer cells from the vial to a sterile centrifuge tube. Add 8-10 ml of pre-warmed *Cell Biologics* Cell Culture Medium.
- Flush the vial with an additional 0.5-1 ml of medium to ensure complete transfer of cells to the centrifuge tube.
- Centrifuge cells at 200 g for 5 minutes.
- Aspirate the supernatant and resuspend the cell pellet in 6 ml of *Cell Biologics'* Cell Culture Growth Medium.
- Recovery cells from cryovial in 10% FBS for the first and second days.
- Add resuspended cells into a T25 flask pre-coated with Gelatin-Based Coating Solution (*Cell Biologics*, Catalog No. 6950).
- Place the T25 flask in a humidified, 5%-CO<sub>2</sub> incubator at 37°C.
- Change culture media the following day to remove non-adherent cells and replenish nutrients.
- Change cell culture medium every day when cells are >70% confluent.
- Cells should be checked daily under a microscope to verify appropriate cell morphology.

### Note:

- You may let cells over-growing for 24-48 h after cells reach confluence before doing any cell testing, cell staining, FACS, or designed experiments.
- Cells can be filtered through a 40 Micron cell strainer (BD 352340) just before passaging cells or doing experiments to remove any clumps of cells and most of the dead/floating large cells.
- Please send us the cell images (>90% confluence) if you have any question or problem with cultured cells.
- Per request, a Certificate of Analysis will be provided for each cell lot purchased.