Mouse Splenocyte Cell Care Manual

INSTRUCTION MANUAL ZBM0090.00

SHIPPING CONDITIONS

Cryopreserved Mouse Splenocyte Cells

Orders are delivered via Federal Express courier.

Must be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Plated cells: Humidified, CO_2 incubator

All Zen-Bio Inc products are for research use only. Not approved for human or veterinary use or for use in diagnostic or clinical procedures.

ORDERING INFORMATION AND TECHNICAL SERVICES

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INTRODUCTION

Mouse splenocytes are dissociated into a single cell suspension so they can be easily manipulated ex-vivo. The splenocytes are isolated from excised spleens by mashing through a 60um screen, resuspending in serum-free medium, and pelleting by centrifugation. Splenocytes are used for a variety of assays including T-cell activation, proliferation in response to mitogens, and cytokine production. Zen-bio offers Mouse splenocyte cells produced at Zen-Bio's facility from normal mouse tissues. Each vial contains a minimum of 25 million viable cells.

PRECAUTIONS

This product is for research use only. *It is not intended for human, veterinary, or in vitro diagnostic use*. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. **Always wear gloves and work behind a protective screen when handling primary human cells.** All media, supplements, and tissue cultureware used in this protocol should be sterile.

Mouse Splenocyte cell viability depends greatly on the use of suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed cell responsiveness in assays may be lower than expected.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

***Note**: Zen-Bio recommends that the Mouse Splenocytes be processed immediately upon receipt.*

- **Cryopreserved Mouse Splenocytes** (catalog # MSP-F)
  Frozen vial containing at least 25 x10^6 mouse splenocytes (store in liquid nitrogen upon receipt)

PREPARATION OF MOUSE SPLENOCYTES FOR INVESTIGATION

1. Remove cells from liquid nitrogen and place immediately into a 37° C water bath and agitate while in bath. Be careful not to submerge the cap of the vial into water. Do not leave the vials in water bath after most of the content has thawed. Rinse the vials with 70% ethanol before taking them to the culture hood.
2. Upon thawing, transfer the cells to a sterile conical bottom centrifuge tube containing 40 ml complete medium. Centrifuge: 400 xg / 20°C / 5 minutes. Aspirate the supernatant. TAKE CARE TO NOT ASPIRATE ANY OF THE CELL PELLET.
3. The cell vial contains a minimum of 25.0 x 10^6 viable cells; however, we recommend performing a cell count to determine a more exact number of cells. Resuspend the cells at 1-10 x 10^6 cells/mL in serum-free DMEM, high glucose.
4. Dilute an aliquot in 0.4% trypan blue solution. We suggest withdrawing an aliquot of 50 µl of cells and mixing with 100 µl of the trypan blue solution, resulting in a dilution factor of 3. Count live (unstained) cells on a hemacytometer.
5. Resuspend the cells at a concentration and in media which are appropriate for the experiment
6. Allow the cells to rest at 37ºC for at least 2-4 hours in media appropriate for the experiment
7. The cells can now be treated with the appropriate stimuli, fixed, and/or stained depending on the intended use of these cells.

FREQUENTLY ASKED QUESTIONS

1. **Are the splenocytes isolated in the presence of FBS?**
   No, serum-free DMEM containing high glucose is used during the isolation

2. **Can these cells tolerate greater than 400 xg centrifugation?**
   Yes, the cells are initially pelleted at 800xg

3. **Have the red blood cells been lysed during preparation of the splenocytes?**
   Yes, using 0.8% NH_4Cl, 0.1 mM EDTA, in water

PATHOGEN TESTING

Our products are tested and are free from mycoplasma contamination. Proper precautions and biological containment should be taken when handling cells, due to their potential biohazardous nature. Always wear gloves and work behind a protective screen when handling primary primate cells.