



Human Splenocyte Cell Care Manual

INSTRUCTION MANUAL ZBM0102.01

SHIPPING CONDITIONS

Human Splenocyte Cells (HSP-F)

Orders are delivered via Federal Express courier. All US and Canada orders are shipped via Federal Express Priority service and are usually received the next day. International orders are usually received in 2-4 days. Please inquire if alternate couriers are needed.

Must be processed immediately upon shipment receipt.

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STORAGE CONDITIONS

Media: +4°C (30 days from ship date); -20°C (3 months from ship date)

Cells: Store in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT.

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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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

HSP-F	CRYOPRESERVED HUMAN SPLENOCYTE CELLS
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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 culture ware used in this protocol should be sterile.

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, expressed or implied, including without limitation implied warranties of merchantability or fitness for a particular purpose, are provided by Zen-Bio, Inc. Zen-Bio, Inc. shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Zen-Bio, Inc. warrants its cells only if Zen-Bio media are used and the recommended protocols are followed without amendment or substitution. Human Splenocytes 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. Splenocytes are to be thawed and used immediately as a feeder layer or in other experiments. A subset of the cells will attach to tissue culture treated cultureware for up to 3 days. However, splenocytes are primarily a suspension culture. Please have your experimental design prepared before thawing the cells. ZenBio, Inc warranty does not include replacement for cells not used in accordance with the manual information.

Contact ZenBio, Inc. within no more than 24 hours after receipt of products for all claims regarding shipment damage, incorrect ordering or other delivery issues. Delivery claims received after 7 days of receipt of products are not subject to replacement or refund.

INTRODUCTION

Human splenocytes are isolated from the excised spleens of consented donors via the gift of organ donation. Each donor has confirmed documentation on file allowing for research use of any non-transplantable organs or tissues. The splenocytes are dissociated into a single cell suspension so they can be easily manipulated ex-vivo. Splenocytes are used for a variety of assays including T-cell activation, proliferation in response to mitogens, and cytokine production. Zen-Bio offers human splenocyte cells produced at Zen-Bio's facility from normal human tissues. Each vial contains 25 million viable cells.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

❖ Cryopreserved Human Splenocyte Cells (Catalog # HSP-F)

- Frozen vial containing a minimum of 25 million viable Human Splenocyte cells.
- Store cells in vapor phase nitrogen (-150°C to -190°C) immediately upon receipt.

❖ Splenocyte Maintenance Medium (Catalog # MSP-1); 100ml

- Complete medium. No additives required.
- Store +4°C or -20°C. (See below)

MEDIUM COMPOSITION

<u>Splenocyte Maintenance Medium (catalog #MSP-1)</u>	<u>Storage and Expiration Date</u>
RPMI-1640 Fetal Bovine Serum (FBS) Heat-inactivated 2-mercaptoethanol L-glutamine Penicillin Streptomycin Amphotericin B	If placed at 4°C upon arrival, the media is stable until the expiration date on the bottle label. If stored at -20°C upon arrival, the media is stable for 3 months . Add fresh antibiotics when you are ready to use. The media will expire 30 days after the thaw date.

THAWING PRIMARY HUMAN SPLENOCYTES _____

IMPORTANT NOTES:

- Human splenocytes will remain primarily as a suspension culture.
 - Some cells will attach to tissue culture treated cultureware for up to 3 days.
 - Please have your experimental design prepared before thawing the cells.
 - ZenBio, Inc warranty does not include replacement for cells not used in accordance with the manual information.
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 at 400xg / 20°C / 5 minutes. Aspirate the supernatant. TAKE CARE TO NOT ASPIRATE ANY OF THE CELL PELLETT.
 3. The cell vial contains a minimum of 25 million viable cells; however, we recommend performing a cell count to determine a more exact number of cells. Resuspend the cells at 1-10 million 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 hemocytometer.
 5. Resuspend the cells at a concentration in media which are appropriate for your experiment
 6. Allow the cells to rest at 37°C 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. Can the splenocytes be expanded?

No. Splenocytes are to be thawed and used immediately in your experiments. A subset of the cells will attach to tissue culture treated cultureware for up to 3 days. However, splenocytes are primarily a suspension culture. Please have your experimental design prepared before thawing the cells. ZenBio, Inc warranty does not include replacement for cells not used in accordance with the manual information.

2. Are the splenocytes isolated in the presence of FBS?

No, serum-free DMEM containing high glucose is used during the isolation.

3. Can these cells tolerate greater than 400xg centrifugation?

Yes, the cells are initially pelleted at 800 x g.

4. Do you test for pathogens? Which ones?

Yes. All tissue donors are screened and found to be negative for HIV-1, HIV-2, Hepatitis B, and Hepatitis C. Frozen samples are screened and found to be negative for HIV-1, HIV-2, Hepatitis B, and Hepatitis C. However, no known test can offer complete assurance that these viruses are not present. Since we cannot test all the pathogens, please treat the culture as a potential infectious reagent at Biosafety Level 2 or higher.

5. Have the red blood cells been lysed during preparation of the splenocytes?

Yes, using 0.8% NH₄Cl, 0.1 mM EDTA, in water.

6. Where are the cells obtained?

The human splenocytes are obtained from the spleens of consented donor cadavers that have been harvested within 24 hours of death.

PATHOGEN TESTING

All tissue donors are screened and found to be negative for HIV-1, HIV-2, Hepatitis B, and Hepatitis C. However, no known test can offer complete assurance that the viruses that these infectious diseases are not present. Since we cannot test all the pathogens, please treat the culture as a potential infectious reagent at Biosafety Level 2 or higher. We recommend using the US Centers for Disease Control (CDC) Universal Precautions for prevention of blood-borne pathogens as a minimum guideline for standards of practice.