Human Bladder Smooth Muscle Cell Care Manual

INSTRUCTION MANUAL ZBM0083.03

SHIPPING CONDITIONS

Human Bladder Smooth Muscle Cells, Cryopreserved

Orders are delivered via Federal Express courier. All USA and Canada orders are shipped via Federal Express Priority service and are usually received the next day. Non North American International orders are usually received in 2-4 days. Primary human cells can be sensitive to extended times at dry ice temperatures. If your transit time will exceed 3 days, please inquire about dry vapor shipper options. Please inquire if alternate couriers are needed.

All orders should be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Media:	+4°C	Expires 30 days from ship date.
	-20°C	Expires 6 months from ship date.

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

Any other use negates the warranty.

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

ORDERING INFORMATION AND TECHNICAL SERVICES

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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

BSM-F	CRYOPRESERVED HUMAN BLADDER SMOOTH MUSCLE CELLS (500,000 CELLS/VIAL)

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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 the performance of cells only if Zen-Bio media are used and the recommended storage conditions and protocols are followed without amendment or substitution. ZenBio, Inc. cryopreserved cells are assured to be viable when stored as recommended and thawed according to Zen-Bio protocols and using the recommended protocol.

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.

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.

Human bladder smooth muscle cell viability depends greatly on the use of suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed, cell growth may be slower than expected.

INTRODUCTION

Smooth muscle cells are found in lymphatic vessels, the walls of blood vessels, the bladder, the uterus, and other areas of the body. The primary function of smooth muscles is to create contractions in the presence of an external stimulus. This stimuli can differ depending on the area of the body to generate individual effects. ATP hydrolysis provides energy for smooth muscle contractions. They are created by a sliding motion between actin filaments and myosin.

Zen-Bio offers bladder smooth muscle cells that were isolated from normal human bladder tissue with a post-mortem interval (PMI) of less than 2 hours in the United States. The smooth muscle cells are isolated by dissecting the outer smooth muscle layer from the inner urothelial cell layer of the bladder prior to explant culturing. Each donor has confirmed documentation on file allowing for research use of the donated tissues. Each vial contains 500,000 viable cells.

QUALITY CONTROL

Human bladder smooth muscle cells are plated at P0 on tissue culture plates and propagated in Bladder Smooth Muscle Cell Growth Medium (cat# BSM-1). The cells are assessed for viability (>80%) and for the presence of the cell surface marker alpha-smooth muscle actin and the smooth muscle-specific protein Calponin.

CATALOG ITEMS

- **Bladder Smooth Muscle Cell Growth Medium**
  - Cat # BSM-1, 500mL
  - Store according to label
- **Cryopreserved Human Bladder Smooth Muscle Cells**
  - Cat # BSM-F
  - Cryopreserved vial containing 500,00 human bladder smooth muscle cells per vial **(store in vapor phase liquid nitrogen IMMEDIATELY upon receipt)** *any other storage negates the warranty*

MEDIUM COMPOSTION

Bladder Smooth Muscle Cell Growth Medium (Cat# BSM-1)	**Storage and Expiration Date**
DMEM, 4.5 g/L (25 mmol/L) D-glucose Fetal Bovine Serum (FBS; USA Origin) Penicillin Streptomycin Amphotericin B	• If stored at 4°C upon arrival, the media is stable until the expiration date on the bottle. • If stored at -20°C upon arrival, the media is stable for 6 months. *The media will expire 30 days after the thaw date.* • Medium is provided ready to use and prepared fresh prior to shipment. **Media is also available without serum and/or phenol red free. Please inquire for custom media requests!**

PLATING AND EXPANSION PROCEDURES

THAWING AND CULTURING HUMAN BLADDER SMOOTH MUSCLE CELLS

Note: Primary human cell viability is greatly dependent on the use of appropriate sterile tissue culture treated cultureware. No extracellular matrix coatings required.

1. Pre-warm Bladder Smooth Muscle Cell Growth Medium (cat# BSM-1]) at 37°C, and prepare all pipets and vessels.
2. Transfer 9.5 mL of warm BSM-1 to a sterile 15 mL conical centrifuge tube.
3. Remove cryovial of human bladder smooth muscle cells from liquid nitrogen and place **immediately** into a 37°C water bath with mild agitation. Be careful not to submerge the cap of the vial into water. For best results, the thawing step should not take more than 1 minute, and should be stopped when there is still visible ice within the vial.
4. Rinse cryovial with 70% ethanol, and wipe cryovial with lint-free lab wiper. Open cryovial under laminar flow hood and resuspend cells in previously prepared 9.5 mL of warmed BSM-1.
5. Centrifuge cell suspension at 400 x *g* for 10 minutes at 20ºC.
6. Carefully aspirate the supernatant, being careful not to disturb the cell pellet, and resuspend in a volume of BSM-1 appropriate for counting the cells. Count cells using a hemocytometer or automated cell counter.
7. The plating density of human bladder smooth muscle cells is 5,000 cells per cm^2 for standard proliferation. Calculate the necessary culture surface area according to the plating density (being sure to reference the manufacturer specifications for cell culture area).
8. Place vessel in an incubator (37°C, 5% CO_2) for cell attachment. Ensure cells are evenly suspended when plating large numbers of plates or flasks. Do not agitate the plates or flasks after plating, making sure the vessel surface is level for even cell distribution.
9. Replace medium after 16-24 hours. Medium should be changed every 3 days until cells reach 80-90% confluency.

OPTIONAL – HUMAN BLADDER SMOOTH MUSCLE CELL SUBCULTURE

Note: Human bladder smooth muscle cells should not be expanded beyond passage 7.

1. Human bladder smooth muscle cells should be sub-confluent (less than 90% confluent) upon harvest for expansion.
2. Pre-warm all reagents and medium to 37ºC, and prepare all pipets and vessels.
3. Carefully aspirate medium from cell culture vessel and wash cells using sterile Dulbecco's phosphate buffered saline without calcium or magnesium (cat# DPBS-1000) to remove all traces of serum, or until there is no foaming of the medium.
4. Remove the DPBS-1000 and release the cells from the cultureware bottom by adding Trypsin/EDTA solution (cat# TRP-100) at 0.1-0.2 mL per cm^2 cultureware surface area.
5. Incubate cells for 5-10 minutes at 37°C if using Trypsin/EDTA.
6. Examine cells under microscope, and once cells begin detaching, gently tap the side of the vessel to loosen the remaining cells.
7. Neutralize Trypsin/EDTA solution using BSM-1 at 0.1-0.2 mL per cm^2 cultureware surface area. Carefully transfer the cell suspension to an appropriate centrifuge tube.

8. Centrifuge cell suspension at 400 x *g* for 10 minutes at 20ºC.

9. Carefully aspirate supernatant, being careful not to disturb the cell pellet, and resuspend the in a volume of BSM-1 appropriate for counting the cells. Count cells using a hemocytometer or automated cell counter.

10. Seed cells at 4,000-10,000 cells per cm^2 (refer to Table 1 below) in the appropriate vessel, and place vessel in an incubator (37°C, 5% CO_2) for cell attachment. Ensure cells are evenly suspended when plating large numbers of plates or flasks. Do not agitate the plates or flasks after plating, making sure the vessel surface is level for even cell distribution.

Table 1: Cultureware Volumes and Cell Density

Cultureware Format	Surface Area	Volume BSM-1	Cells/Cultureware Unit
25cm^2 flask	25cm^2	5 mL	100,000-250,000
75cm^2 flask	75 cm^2	13 mL	300,000-750,000
225cm^2 flask	225 cm^2	30 mL	900,000-2,250,000
1-layer cell factory	632 cm^2	1500 mL	2.53 Million

11. Replace the medium every 2-3 days. Once cells have reached 80-90% confluency they should be subcultured further or harvested and cryopreserved.

CRYOPRESERVATION PROCEDURE

1. Human bladder smooth muscle cells should be sub-confluent (less than 90% confluent) upon harvest for cryopreservation.
2. Aspirate medium and wash cells using sterile Dulbecco's phosphate buffered saline without calcium or magnesium (cat# DPBS-1000) to remove all traces of serum, or until there is no foaming of the medium.
3. Remove the DPBS-1000 and release the cells from the cultureware bottom by adding Trypsin/EDTA solution (cat# TRP-100) at 0.1-0.2 mL per cm^2 cultureware surface area.
4. Incubate cells for 5-10 minutes at 37°C if using Trypsin/EDTA.
5. Examine cells under microscope, and once cells begin detaching, gently tap the side of the vessel to loosen the remaining cells.
6. Neutralize Trypsin/EDTA solution using Bladder Smooth Muscle Cell Growth Medium (cat# BSM-1) at 0.1-0.2 mL per cm^2 cultureware surface area. Carefully transfer the cell suspension to an appropriate centrifuge tube.
7. Centrifuge cell suspension at 400 x *g* for 10 minutes at 20ºC.
8. Carefully aspirate supernatant, being careful not to disturb the cell pellet, and resuspend the in a volume of BSM-1 appropriate for counting the cells. Count cells using a hemocytometer or automated cell counter.
9. Centrifuge cell suspension at 400 x *g* for 10 minutes at 20ºC.
10. Carefully aspirate supernatant, being careful not to disturb the cell pellet, and suspend in cold CryoStor® CS10 at a concentration of 1 million cells per mL. Do not exceed a 6:1 ratio of cells (per million) to volume cryopreservation medium (per mL). Remember to account for the volume of the cell pellet before adding the volume of cryopreservation medium necessary for cell suspension.
11. If using a controlled-rate freezer: Freeze by reducing the temperature 1°C per minute until the temperature reaches -80° C. If using a cell cryopreservation container, prepare according to the manufacturer's instructions.
12. For best results we recommend transferring the vials to the vapor phase of a liquid nitrogen storage facility as soon as possible after the cells have reached -80°C.

TROUBLESHOOTING GUIDE

Observation	Possible Cause(s)	Suggestion(s)
Bladder smooth muscle cells do not grow.	1. Cells have been passaged too many times. 2. Cells Expanded too high.	1. Use cells of a lower passage number. 2. Do not exceed a 1:5 expansion ratio.
Edge effects.	• Medium in outside wells evaporated.	• Ensure a saturated humidity in the incubator. • Feed the cells at least every 3 days. Do not exceed 3 days between feeding. • Make sure multiple plates are not stacked more than 3 plates high.

FREQUENTLY ASKED QUESTIONS

Can I expand these cells and what is the maximum passage if so?

Yes. The human smooth muscle bladder cells are sold at passage 2, 3 or 4. Passage 7 is the maximum recommended expansion of the cells.

What is the average doubling time of these cells?

Average doubling time ranges from 24-48 hours. However, keep in mind that the replication rate varies slightly from donor to donor.

Are antibiotics included in the medium?

Yes. Penicillin, streptomycin and amphotericin B are included in the medium.

PATHOGEN TESTING

Samples from each donor are tested via PCR and found non-reactive to viral DNA from HIV and Hepatitis B and viral RNA from Hepatitis C. However, no known test can offer complete assurance that these viruses are not present. Since we cannot test all pathogens, always treat the culture as a potentially infectious reagent. 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. Our products are tested for mycoplasma contamination. Mycoplasma is not detected in our labs. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. All human based products should be handled at a BSL-1 (Biosafety Level 1) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.