



Human Bladder Smooth Muscle Cell Care Manual

INSTRUCTION MANUAL ZBM0083.00

SHIPPING CONDITIONS

Cryopreserved Human Bladder Smooth Muscle Cells

Orders are delivered via Federal Express courier.

Must be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Media: Store at 2-8°C

Plated cells: Humidified, CO₂ 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

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 in different areas 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.

The outer smooth muscle layer is dissected away from the inner urothelial cell layer of the bladder prior to explant culturing to isolate the smooth muscle cells. These cells express the smooth muscle cell markers smooth muscle alpha-actin and calponin, and are functional as demonstrated by a cell contraction assay. Zen-Bio offers smooth muscle cells produced at Zen-Bio's facility from normal human bladder tissues with a post moretem interval less than 2 hours. Each vial contains a minimum of 500,000 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.

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 responsiveness in assays may be lower than expected.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- Cryopreserved human bladder smooth muscle cells (Cat # BSM-F)
- 50ml Smooth Muscle Cell Medium

MEDIUM COMPOSITION

Bladder Smooth Muscle Cell Growth Medium (cat# BSM-1)

DMEM
Fetal bovine serum
Penicillin
Streptomycin
Amphotericin B

NOTE:

All media are provided ready to use.
All Zen-Bio, Inc media are also available phenol red free.
Please inquire for custom media requests.

PLATING PROCEDURES

1. Remove cells from liquid nitrogen and place immediately into a 37°C water bath with agitation. 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 the thawing, add the cells to a sterile conical bottom centrifuge tube, containing 9 ml of Bladder Smooth Muscle Cell Growth Medium (cat# BSM-1).
3. Centrifuge at 400 x g, 20°C, 10 minutes. Aspirate the medium and resuspend cells in a volume of DF-1 appropriate for counting the cells. Count using a hemacytometer.
4. Place approximately 5,000 cells/cm² using BSM-1 medium.
5. Maintain the cells in a humidified 37°C incubator using 5% CO₂.

EXPANSION OF HUMAN BLADDER SMOOTH MUSCLE CELLS

1. Aspirate the medium from the culture vessels containing cells
2. Wash cells by adding appropriate amount of PBS and gently swirl the culture vessel
3. Aspirate PBS
4. Add appropriate amount of 0.25% Trypsin/EDTA per culture vessel
5. Incubate cells with Trypsin 5-10 minutes, wash cells, and collect the cells into an appropriate size centrifuge tube with the serum-containing medium that is least 10% of the collected volume to neutralize the trypsin
6. Centrifuge cells to pellet and resuspend the pellet with culture medium

7. Count and plate at seeding density of 4,000-10,000cells/cm² (See chart below)

Table 1.

Cultureware	Surface area	Volume Medium (ml)	Cells/unit
25cm ² flask	25cm ²	5	100,000-250,000
75cm ² flask	75 cm ²	13	300,000-750,000
225cm ² flask	225 cm ²	30	900,000-2,250,000
1-layer cell factory	632 cm ²	1500	2.53 X 10 ⁶

8. Add resuspended cells to appropriate amount with medium and plate as per culture vessel (see Table 1 above)
9. Return culture vessel to a humidified 37°C incubator in 5% CO₂.

FREQUENTLY ASKED QUESTIONS ---

1. **Can I expand these cells?**

a. Yes.

2. **At what passage are the cells sold? What is the maximum passage?**

The human smooth muscle bladder cells are sold at passage 2, 3 or 4. Passage 9 is the maximum recommended expansion of the cells.

3. **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.

4. **Are antibiotics included in the medium?**

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

PATHOGEN TESTING ---

Samples from each donor are tested to assess reactivity for HIV-1, HIV-2, hepatitis B, hepatitis C. However, no known test can offer complete assurance that the cells are pathogen free. Our products are tested and are free from mycoplasma contamination. 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-2 (Biosafety Level 2) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.