

**P10751-IM**

Human Small Intestine Smooth Muscle Cells (HSISMC) provided by Innoprot are isolated from healthy human small intestine tissue and represent a reliable in vitro model for gastrointestinal research. Smooth muscle contraction plays a key role in regulating gastrointestinal motility, a complex physiological process essential for proper digestion and nutrient absorption. Although the biochemical mechanisms underlying excitation-contraction coupling in smooth muscle are not yet fully elucidated, it is well established that cytosolic  $Ca^{2+}$  acts as a critical signaling molecule in this process. In pathological conditions such as intestinal inflammation, smooth muscle layers often become thickened due to increased expression of smooth muscle-specific actins, contributing to altered motility and tissue remodeling.

The availability of HSISMC in culture provides a valuable and reproducible tool for studying the contractile, proliferative, and connective tissue responses of human intestinal smooth muscle. This model supports the advancement of research into gastrointestinal physiology and disease, as well as the development of targeted therapies.



**IMMORTALIZED HUMAN  
SMALL INTESTINE SMOOTH MUSCLE CELLS**

**Product Type:** Immortalized Human Small Intestine Smooth Muscle Cells

**Catalog Number:** P10751-IM

**Immortalization:** SV40 Large T Antigen. G418 resistant.

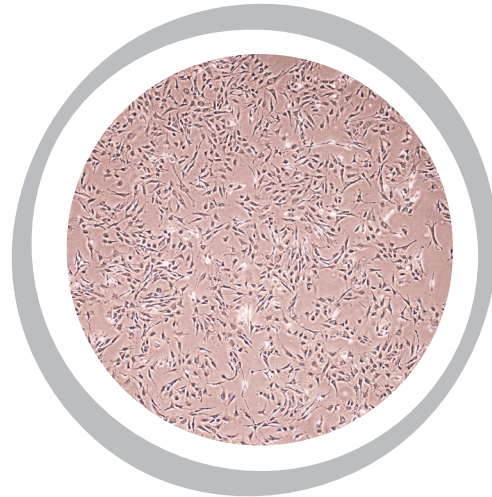
**Number of cells:**  $>1 \times 10^6$  cells (cryopreserved vials)

**Storage:** Liquid Nitrogen

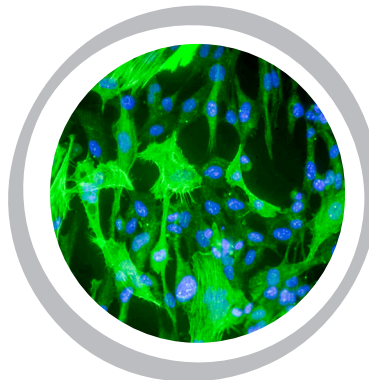
**Source:** Human Healthy Small Intestine

**Recommended Medium:** Smooth Muscle Cell Medium (Ref.: P60125)

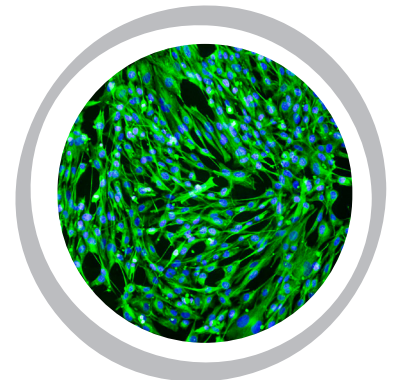
**Product Characterization:** Immunofluorescent method  $\alpha$ -Smooth Muscle Actin and Vimentin. Functional assay: Acetylcholine-Induced calcium mobilization.



Phase-contrast



$\alpha$ -SMA



Vimentin

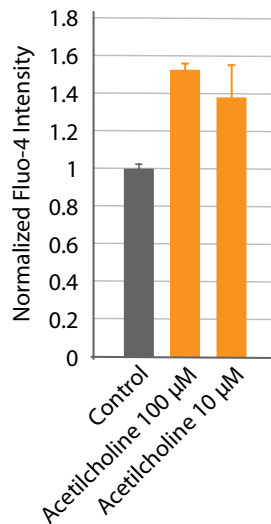
**Use Restriction:** This product contains a proprietary nucleic acid coding for a proprietary fluorescent protein intended to be used for research purposes only. No rights are conveyed to modify or clone the gene encoding fluorescent protein contained in this product, or to use the gene or protein other than for non-commercial research, including use for validation or screening compounds. For information on commercial licensing, contact Licensing Department, Evrogen JSC, email: [license@evrogen.com](mailto:license@evrogen.com)

## About Immortalized Human Small Intestine Smooth Muscle Cell Line

The immortalized human small intestine smooth muscle cell line has been developed through genetic modification of primary human small intestine cells, employing the SV40LT protein as the immortalization method. The SV40LT protein, derived from the Simian Virus 40 Large T-antigen, has been introduced to confer immortality to the cells, allowing for an extended lifespan compared to primary cells that typically undergo senescence after a limited number of passages. The use of SV40LT for immortalization is a common technique in cell biology and allows for the establishment of cell lines with a more stable and prolonged growth capacity. This modification enables researchers to conduct long-term experiments and studies that require consistent cellular behavior over an extended period. Primary cells exhibit senescence following the 5th passage, whereas the SV40LT-transduced cells demonstrate a prolonged viability, extending beyond 20 passages.

## Functional assay

A functional assay was conducted to evaluate the responsiveness of immortalized small intestinal smooth muscle cells to acetylcholine, a key neurotransmitter involved in gastrointestinal motility. Cells were exposed to acetylcholine, and intracellular calcium levels were measured using Fluo-4 to assess their activation upon cholinergic stimulation. A clear increase in intracellular calcium concentration was detected, indicating that the cells exhibit a positive functional response to acetylcholine.



## Culturing conditions

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### 1 IMMEDIATELY UPON DELIVERY

- 1.1 Remove the vial from the shipping container to check for freezing.
- 1.2 Transfer the frozen vial to liquid nitrogen until ready to thaw.

### 2 THAWING CELLS:

- 2.1 Prepare "Thawing medium" by combining 500 ml of basal medium, 25 ml of fetal bovine serum, 5 ml of Growth supplement and 5 ml of penicillin/streptomycin solution.
- 2.2 Thaw cells rapidly in a 37°C water bath; avoid allowing the sample to warm to 37°C. Cryovials should be cool to the touch when removed.
- 2.3 Remove the vial, wipe it dry, and transfer it to a sterile field.
- 2.4 Rinse the vial with 70% ethanol, then wipe to remove excess. Open the vial and resuspend its contents using a 1 ml Eppendorf pipette.
- 2.5 Dispense the contents into a 25 cm<sup>2</sup> culture flask with warm complete media (FBS percentage can be increased up to 10% for better culture establishment).
- 2.6 Place the flask in the incubator.
- 2.7 For optimal results, avoid disturbing the culture for 16 hours after initiation. Change the growth medium the next day to remove unattached cells, then every other day thereafter.

### 3 MAINTENANCE OF THE CULTURE:

- 3.1 Change medium 48 hours after establishing a subculture.
- 3.2 Subculture when cells are over 90% confluent.

### 4 SUBCULTURING:

Remove medium, rinse with 0.05% trypsin-EDTA solution. Add 1 to 2 mL of trypsin-EDTA solution and allow the flask to sit until cells detach. Add fresh culture medium, aspirate, and dispense into new culture flasks. Recommended subcultivation ratio of 1:2 to 1:6. Medium Renewal: 2 to 3 times per week. Reagents for cryopreservation: Cryostor S10.

## Quality Control / Biosafety

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The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi.

### THIS PRODUCT IS FOR RESEARCH PURPOSES ONLY

It is not to be used for drug or diagnostic purposes, nor is it intended for human use. Innoprot products may not be resold, modified for resale, or used to manufacture commercial products without written approval of Innovative Technologies in Biological Systems, S.L.