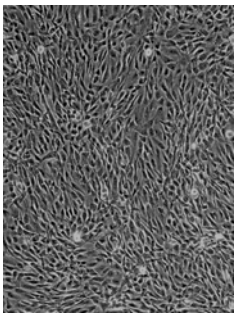
REF: P10796

MALE REPRODUCTIVE CELL SYSTEM INNOPROFILE™

HUMAN SERTOLI CELLS



Product Type:	Cryo-preserved Sertoli Cells
Catalog Number:	P10796
Source:	Human Testis
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

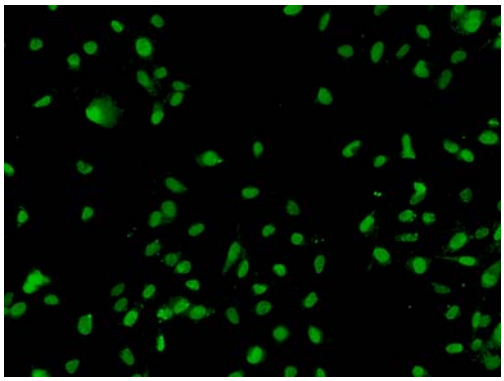
Human Sertoli Cells (HSerC) provided by Innoprot are isolated from human tesis. HSerC are cryopreserved at passage one and delivered frozen. HSerC are guaranteed to further expand for 15 population doublings in the conditions provided in the technical sheet.

Sertoli Cells (SerC) are essential for testicular development, spermatogenesis, and formation of the blood-testis barrier. SerC limit the passage of substances such as hormones and nutrients to the adluminal compartment of the seminiferous tubules.. Aberrant SerC proliferation can contribute to the development of male reproductive disorders such as testicular germ-cell cancer, cryptorchidism, hypospadias, and low sperm count. SerC proliferation is in part controlled by follicle-stimulating hormone (FSH) and thyroid hormone (TH), where FSH drives proliferation and TH promotes a more quiescent state. Cultured Human SerC are a useful in vitro model to understand testicular dysgenesis syndrome and to develop treatments for male reproductive disorders.

Recommended Medium

- Sertoli Cell Medium

  (Reference: P60139)



Product Characterization

Immunofluorescent method

- Sox-9
- GATA-4

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

Product Use

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures

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INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: Cryopreserved cells are very delicate. Thaw the vial in a 37 ºC waterbath and return them to culture as quickly as possible with minimal handling!

Set up culture after receiving the order:

1. Prepare a poly-L-lysine coated flask (2 µg/cm2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 150 µl of poly-L-lysine stock solution (1 mg/ml). Leave the flask in incubator overnight (minimum one hour at 37ºC incubator).

2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the tube with medium to recover the entire volume.

3. Rinse the poly-L-lysine coated vessel with sterile water twice and then add 15 ml of complete medium. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.

4. Place the frozen vial in a 37ºC water bath. Hold and rotate the vial gently until the contents completely thaw. Remove the vial from the water bath promptly, wipe it down with 70% ethanol and transfer it to a sterile field.

5. Remove the cap carefully without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessel. A seeding density of 5,000 cells/cm^2 is recommended.

Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that HSerC are plated in poly-L-lysine coated flask that promotes cell attachment and growth.

6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.

7. Return the culture vessels to the incubator.

8. For the best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells.

2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.

3. Once the culture reaches 70% confluence, change medium every other day until the culture is approximately 90% confluent

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Subculture:

1. Subculture when the culture reaches 90% confluency or above.

2. Prepare poly-L-lysine-coated culture vessels (2 µg/cm^2) one day before subculture.

3. Warm complete medium, trypsin/EDTA solution, Trypsin Neutralization Solution, and DPBS (Ref: P60305 - Primary Cells Subculturing Kit) to room temperature. We do not recommend warming reagents and medium in a 37ºC water bath prior to use.

4. Rinse the cells with DPBS.

5. Add 8 ml of DPBS and then 2 ml of T/E solution into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37ºC incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.

6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).

7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37ºC for another 1 to 2 minutes (no solution in the flask at this moment).

8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.

9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.

10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.

11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.

12. Count and plate cells in a new poly-L-lysine-coated culture vessel with the recommended cell density.

Caution: *Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].*

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. *J Tissue Culture Methods*. 11(4).

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