Human Sertoli Cells (HSerC)

Catalog #4520

Cell Specification

Sertoli Cells (SerC) are essential for testicular development, spermatogenesis, and formation of the blood-testis barrier [1, 2]. SerC limit the passage of substances such as hormones and nutrients to the adluminal compartment of the seminiferous tubules [1]. In addition to forming the blood-testis barrier, SerC also provide the main structural support for the seminiferous tubules and protect the germ cells from the immune system [1]. Aberrant SerC proliferation can contribute to the development of male reproductive disorders such as testicular germ-cell cancer, cryptorchidism, hypospadias, and low sperm count [2]. 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 [3]. Cultured Human SerC are a useful *in vitro* model to better understand testicular dysgenesis syndrome and to develop treatments for male reproductive disorders.

HSerC from ScienCell Research Laboratories are isolated from human testis. HSerC are cryopreserved at passage one and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HSerC are characterized by immunofluorescence with antibody specific to GATA-4 and Sox-9. HSerC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HSerC are guaranteed to further expand for 15 population doublings under the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Sertoli Cell Medium (SerCM, Cat. #4521) for the culturing of HSerC *in vitro*.

Product Use

HSerC are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[1] Chui K, Trivedi A, Cheng C, Cherbavaz, Dazin P, Huynh A, Mitchell J, Rabinovich G, Noble-Haeusslein L, John C. (2011) “Characterization and functionality of proliferative human Sertoli cells.” *Cell Transplant*. 20(5): 619-635.

[2] Sharpe R, McKinnell C, Kivlin C, Fisher J. (2003) “Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood.” *Reproduction*. 125: 769-784.

[3] Tarulli G, Stanton P, Meachem S. (2012) “Is the adult Sertoli cell terminally differentiated? *Biol Reprod*. 87(1): 1-11.

Instructions for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C water bath and return the cells to culture as quickly as possible with minimal handling!

Initiating the culture:

1. Prepare a poly-L-lysine coated culture vessel (2 μg/cm^2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15 μl of poly-L-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in incubator overnight (minimum one hour at 37°C 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 37°C 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 residual DMSO in the culture. It is also important that cells are plated in poly-L-lysine coated culture vessels to promote cell attachment.
6. Replace the cap or lid, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to allow gas exchange.
7. Return the culture vessel 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.

Maintaining the culture:

1. Refresh supplemented culture 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% confluency, change medium every other day until the culture is approximately 90% confluent.

Subculturing:

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 (T/E, Cat. #0103), T/E neutralization solution (TNS, Cat. #0113), and DPBS (Ca^{++}- and Mg^{++} -free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a 37°C 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 37°C 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 37°C 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%.

    Note: Use ScienCell T/E solution that is optimized to minimize cell damages due to over trypsinization.

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 biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with 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 WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Culture Methods*. 11: 191-9.