Rev. 1

Human Leydig Cells (HLC)

Catalog #4510

Cell Specification

Leydig Cells (LC) are located in the interstitium of the testis near the seminiferous tubules [1, 2]. One of the main functions of LC is to produce testosterone after stimulation by pituitary luteinizing hormone [1, 2, 3]. LC, through synthesis of testosterone, play a key role in mammalian sex determination by inducing differentiation of the Wolffian ducts into male reproductive organs [3]. Additionally, LC produce insulin-like growth factor 3 which is required for scrotal descent of the testis [1]. Recent studies have indicated that molecules such as cyclic nucleotide phosphodiesterases can modulate testosterone production in LC, suggesting that testosterone synthesis may be manipulated using pharmacological targeting [2]. Cultured Human LC are a useful *in vitro* model to better understand testicular development and to develop treatments for male reproductive disorders.

HLC from ScienCell Research Laboratories are isolated from human testis. HLC are cryopreserved at passage one and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HLC are characterized by immunofluorescence with antibody specific to GATA-4 and CK-18. HLC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HLC are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; however, *HLC are not recommended for expanding or long-term cultures due to limited expansion capacity.*

Recommended Medium

It is recommended to use Leydig Cell Medium (LCM, Cat. #4511) for the culturing of HLC *in vitro*.

Product Use

HLC 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] Chen H, Ge R, Zirkin B. “Leydig cells: From stem cells to aging.” *Mol Cell Endocrinol.* 306(1-2): 9-16.
[2] Vasta V, Shimizu-Albergine M, Beavo J. “Modulation of Leydig cell function by cyclic nucleotide phosphodiesterase 8A.” *Proc Natl Acad Scie USA*. 103(52): 19925-19930.
[3] Lejeune H, Habert R, Saez JM. “Origin, proliferation and differentiation of Leydig cells.” *J Mol Endocrinol*. 20(1): 1-25.

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Instructions for culturing primary cells

Caution: Cryopreserved primary 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! Do not centrifuge the cells after thawing as this can damage the cells.

Note: *HLC are very sensitive cells and they are not expected to proliferate many times in culture. Experiments should be well organized before thawing the cells and cells should be used as soon as possible for experiments.*

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37°C, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare a poly-L-lysine-coated culture vessel (2 μg/cm^2, T-75 flask is recommended). To obtain a 2 μg/cm^2 poly-L-lysine-coated culture vessel, 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 a 37°C incubator overnight (or for a minimum of one hour).
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add 20 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. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-L-lysine-coated culture vessel.

   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 of the culture vessel 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. Do not disturb the culture for at least 16 hours after initiation. Refresh culture medium the next day to remove residual DMSO and unattached cells.

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, 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.
4. Use cells promptly for experiments.

   Note: We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

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 Cult Methods*. 11: 191-9.