Rev. 1

Human Villous Trophoblasts (HVT)

Catalog #7120

Cell Specification

The trophoblast begins at the outer covering of the early blastocyst and provides the route of nourishment between the maternal endometrium and the developing embryo. The trophoblast adhesion to the uterine wall is the requisite first step of implantation and, subsequently, placentation. Human villous tryophoblasts (HVT) covering the villi of the placenta provide the surface for the exchange of oxygen and nutrients with the maternal circulation. They synthesize and release chorionic gonadotropin, placental lactogen and angiogenin [1] and express CXCR4, CCR5 and prolactin gene family [2, 3]. They acquire CCR1 as they differentiate to an invasive phenotype at the villous-anchoring sites [4]. The features of HVT, together with the recent establishment of trophoblast stem cells, make them an ideal genetic platform to study cell differentiation and organogenesis.

HVT from ScienCell Research Laboratories are isolated from human placental villi. HVT are cryopreserved at passage one and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. HVT are characterized by HCG ELISA. HVT are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HVT are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; however, *HVT are not recommended for expanding or long-term cultures since the cells do not proliferate in regular culture*.

Recommended Medium

It is recommended to use Trophoblast Medium (TM, Cat. #7121) for the culturing of HVT *in vitro*.

Product Use

HVT 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] Pavlov N, Hatzi E, Bassagliam Y, Frendo JL, Brion DE, Badet J. (2003) "Angiogenin distribution in human term placenta, and expression by cultured trophoblastic cells." *Angiogenesis*. 6(4):317-30.

[2] Maldonado-Estrada J, Menu E, Roques P, Vaslin B, Dautry-Varsat A, Barre-Sinoussi F, Chaouat G. (2003) "Predominant intracellular expression of CXCR4 and CCR5 in purified primary trophoblast cells from first trimester and term human placentae." *Am J Reprod Immunol*. 50(4):291-301.

[3] Wiemers DO, Ain R, Ohboshi S, Soares MJ. (2003) "Migratory trophoblast cells express a newly identified member of the prolactin gene family." *J Endocrinol*. 179(3):335-46.

[4] Sato Y, Higuchi T, Yoshioka S, Tatsumi K, Fujiwara H, Fujii S. (2003) "Trophoblasts acquire a chemokine receptor, CCR1, as they differentiate towards invasive phenotype." *Development*. 130(22):5519-32.

Instructions for culturing cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC 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: *Experiments should be well organized before thawing HVT. It is recommended that HVT are used for experiments as quickly as possible after thawing the cells.* ***HVT cannot be subcultured or passaged, as the cells do not proliferate.***

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37ºC, 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 plate (2 µg/cm^2 is recommended). For example, add 2 ml of sterile water to one well of a 6-well plate and then add 20µl of poly-$_L$-lysine stock solution (1 mg/ml, Cat. #0403). Leave the plate in a 37ºC incubator overnight (or for a minimum of one hour).
2. Prepare complete medium (TM, Cat. #7121). Thaw TGS (Cat. #7152), FBS (Cat. #0025) and P/S solution (Cat. #0503) at 37ºC. Gently tilt the tubes several times to ensure the contents are completely mixed before adding to the medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. In a sterile field, remove the caps without touching the interior threads with fingers. Add TGS, FBS and P/S solution to the medium and mix well.
3. Rinse the poly-$_L$-lysine-coated culture vessel twice with sterile water and then add the volume of complete medium recommended in Table 1 or Table 2. Leave the plate(s) 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. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.

   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.*
5. Carefully remove the cap without touching the interior threads and gently resuspend the cell suspension. A seeding density of 10,000-20,000 cells/cm^2 is recommended depending on your experiments. We recommend following Table 1 for seeding HVT onto 6-well, 12-well, or 24-well plates. For seeding HVT on 60 mm plates, use Table 2.

Table 1
Recommended cell suspension volume per vial using a 6-well, 12-well, or 24 well format

Well format	Surface area/well (approx. values)	Volume of media/well	Volume of cell suspension from vial/well	# of wells/vial
6-well	9.6 cm^2	3.0 ml	150 µl	6 wells
12-well	3.9 cm^2	2.0 ml	60 µl	15 wells
24-well	1.9 cm^2	1.0 ml	30 µl	30 wells

Table 2
Recommended cell suspension volume per vial using 60 mm plates

Plate Format	Surface area/plate (approx. values)	Volume of cell suspension from vial/plate	# of plates/vial	Volume of media (ml)/plate
60 mm	21 cm^2	300 µl	3	3.0 ml

6. Pipet the correct volume of cell suspension into each well of an equilibrated, poly-L-lysine-coated culture plate containing complete medium. Replace the lid of the culture plate and gently rock the plate to distribute the cells evenly.
7. Return the culture plate to the incubator.
8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the culture medium in 24 hours to remove residual DMSO and unattached cells.
9. 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