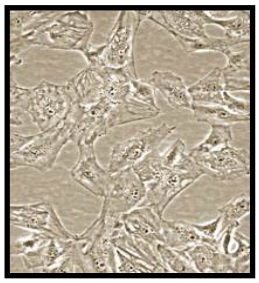
REF: P10958

REPRODUCTIVE CELL SYSTEM INNOPROFILE™

HUMAN VILLOUS TROPHOBLASTS



Product Type:	Cryo-preserved Villous Trophoblasts
Catalog Number:	P10958
Source:	Placental Villi
Number of Cells:	1 x 10^6 Cells / vial (1ml)
Storage:	Liquid Nitrogen

Human Villous Trophoblasts (HVT) provided by Innoprot are isolated from human placental villi. HVT are cryopreserved at passage primary culture and delivered frozen. HVT are guaranteed to further culture under the conditions provided in this data sheet; however, HVT are not recommended for expanding or long-term cultures since the cells do not proliferate in regular culture.

The trophoblast begins as 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 nutrient with the maternal circulation. They synthesize and release chorionic gonadotropin, placental lactogen and angiogenin; expression of CXCR4, CCR5 and prolactin gene family. They acquire CCR1 as they differentiate to an invasive phenotype at the villous-anchoring sites.

Recommended Medium

- Trophoblast Medium

  (Reference: P60128)

Product Characterization

ELISA method

- HCG ELISA

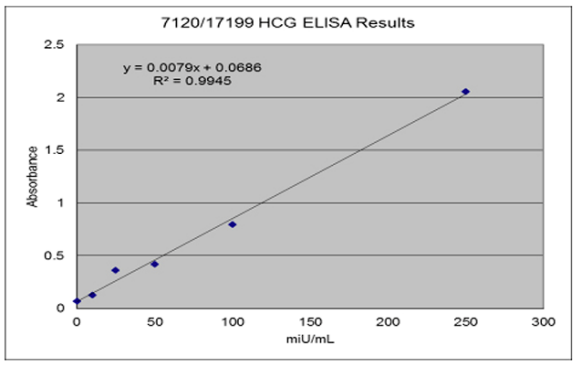


Fig.2. HCG ELISA results. HVT after isolation 375.62 mIU/ml, HVT after freeze/thaw 29.92.

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°C waterbath and return them to culture as quickly as possible with minimal handling!

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.

Set up culture after receiving the order:

1. Prepare a poly-L-lysine-coated culture plate (2 µg/cm2 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. #PLL). Leave the plate in a 37°C incubator overnight (or for a minimum of one hour).

2. Prepare complete medium (TM). Thaw TGS, FBS and P/S solution at 37°C. 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 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.

   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.

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.

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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

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