

Protocol for passaging of TB/SVERT350

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Version: February 2025

Evercyte Ord. No.:	CLHT-070-0350
Designation:	TB/SVERT350, human trophoblasts
Growth medium:	<p>TrophoUp medium contains the herein described components and is prepared as described in the following:</p> <p>80% TS medium (see “<i>Protocol preparation of TS medium</i>”, stored at 4°C) 20% IMDM-FBS medium (see “<i>Protocol preparation of IMDM-FBS medium</i>”, stored at 4°C) 100 µg/ml G418 (Invivogen, Cat# ant-gn-5, stored at 4°C)</p> <ul style="list-style-type: none">- take 40 ml of TS medium (ready-to-use)- add 10 ml of IMDM-FBS medium (ready-to-use)- add 50 µl of G418 stock solution (100 mg/ml, ready-to-use)- mix properly- store at 4°C for a maximum of 4 weeks- temper the medium to room temperature (not 37°C) before use
Coating:	<p>3 mg/ml Collagen I solution</p> <p>The coating solution is prepared by dissolving the lyophilized Collagen I powder in 0.01 N HCl. The following reagents are needed for preparation of Collagen I coated cell culture flasks:</p> <p>Collagen I (Stemcell Technologies, Cat# 07005, 15 mg, stored at -20°C) 25% HCL (Emsure, Merck, Cat# 1.00316.1011, stored at RT) Hypure Cell Culture grade Water (Cytiva, Cat# SH30529, stored at RT) Phosphate buffered saline PBS (Gibco, Cat# 14190-144, stored at RT)</p> <p>For preparation of 100 ml 0.01 N HCl stock solution mix the following components:</p> <ul style="list-style-type: none">- take 50 ml HyPure Cell Culture Grade Water (ready-to-use)- add 133,3 µl 25 % HCl (ready-to-use)- add 50 ml Hypure Cell Culture Grade Water (ready-to-use)- sterile filter with a 0.22 µm filter <p>For preparation of Collagen I stock solution (3 mg/ml, 200 x) mix the following components:</p> <ul style="list-style-type: none">- add 5 ml of 0.01 N HCl to 15 mg of lyophilized Collagen I powder- mix gently until everything has been solubilized

- store at 4°C until use up to 3 months

For coating one T25 roux flask proceed as follows:

- pipette 2 ml of 0.01 N HCl to a sterile tube, add 10 µl of Collagen I stock solution (3 mg/ml) and mix gently
- transfer the 2 ml of 1x Collagen I solution to a T25 roux flask
- completely wet the surface of the culture flask
- incubate at room temperature for at least 1 hour
- remove excess of Collagen I solution
- wash the roux flask once with PBS (160 µl/cm²)
- remove PBS completely
- use culture flask immediately for seeding of cells, the surface must not to dry out

Additional reagents:	Phosphate buffered saline (PBS) (Gibco, Cat# 14190-144, ready-to-use, stored at RT) Trypsin-Inhibitor (Gibco, Cat# R007100, ready-to-use, stored at 4°C) Trypsin-EDTA (Gibco, Cat# 25300054, ready-to-use, stored at 4°C) Trypsin 0.25% (Trypsin 2.5% Gibco, Cat# 15090046, diluted 1:10 in PBS, stored at 4°C)
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Passaging of cells:	<ul style="list-style-type: none"> - remove and discard the culture medium - wash the cells twice with PBS (each 160 µl/cm²), remove PBS completely - add 1:1 Trypsin-EDTA and Trypsin 0.25% (10 µl/cm² of each solution), make sure that all cells have been in contact with this solution - incubate the culture flask at 37°C for approximately 10-15 minutes - observe cell detachment under an inverted microscope - as soon as all cells are detached (if necessary, agitate the cells by gently hitting the flask), add same amount Trypsin-Inhibitor (20 µl/cm²) - add growth medium (about 160 µl/cm²) - centrifuge at 180 g for 5 min - discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 160 µl/cm²) - transfer appropriate aliquots of the cell suspension to Collagen I pre-coated culture flasks supplemented with growth medium (final volume of 240 µl/cm²) - a split ratio of 1:4 to 1:6 1-2 times a week is recommended (after having reached about 80-90 % confluence) - cultivate cells at 37°C in a humidified atmosphere with 5% CO₂
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Related products:	<ul style="list-style-type: none"> - TrophoUp medium (Evercyte, Cat# MHT-070), ready-to-use medium - TB/SVTER350, human trophoblasts (Evercyte, Cat# CLHT-070-0350)
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