

Protocol for preparation of ProxUp2 medium

Version: January 2023

Evercyte Ord. No.: MHT-003-2

Reagents: The ProxUp2 medium for cultivation of RPTEC/TERT1 cells can either be ordered from Evercyte as ready-to-use medium (Cat# MHT-003-2) or as basal medium (Cat# MHT-003-2-B) plus supplements (Cat# MHT-003-2-S).

The medium can also be prepared by mixing the following components:

DMEM/F12 (1:1) (PAN-Biotech, Cat# P04-41154)
10 mM HEPES-buffer (Sigma-Aldrich, Cat# H0887, ready-to-use)
0.5 % FBS (PAN-Biotech, Cat# P30-3031, ready-to-use, stored at 4°C after thawing)
10 ng/ml hEGF (Sigma-Aldrich, Cat# E9644)
5 pM 3,3',5-Triiodo-L-thyronine sodium salt (T3, Sigma-Aldrich, Cat# T6397)
3.5 µg/ml L-Ascorbic Acid (Sigma-Aldrich, Cat# A4544)
5 µg/ml Transferrin Holo (Merck Millipore, Cat# 616424)
25 ng/ml Prostaglandine E1 (Sigma-Aldrich, Cat# P8908)
25 ng/ml Hydrocortisone (Sigma-Aldrich, Cat# H0396)
8.65 ng/ml Sodium-Selenite (Sigma-Aldrich, Cat# S5261)
5 µg/ml Insulin (Sigma-Aldrich, Cat# I9278, ready-to-use)
100 µg/ml G418 (InvivoGen, Cat# ant-gn-5, ready-to-use)

- take one bottle of DMEM/F12 (1:1) (500 ml)
- add 5 ml of Hepes (1M, ready-to-use)
- add 2.5 ml of FBS (ready-to-use)
- add 250 µl of hEGF stock (20 µg/ml, prepared in cell culture grade water)
- add 250 µl of T3 stock (10 nM, prepared in NaOH, PBS)
- add 250 µl of Ascorbic acid stock (7 mg/ml, prepared in cell culture grade water)
- add 250 µl of Transferrin Holo stock (10 mg/ml, prepared in cell culture grade water)
- add 250 µl of Prostaglandine E1 stock (50 µg/ml, prepared in basal medium)
- add 250 µl of Hydrocortisone stock (50 µg/ml, prepared in cell culture grade water)
- add 250 µl of Sodium-Selenite stock (100 µM, prepared in cell culture grade water)
- add 250 µl of Insulin (10 mg/ml, ready-to-use)
- add 500 µl of G418 stock (100 mg/ml, ready-to-use)
- mix properly
- store at 4°C for 4 weeks
- temper the medium to room temperature (not 37°C) before use

Related products: RPTEC/TERT1 (Evercyte, Cat# CHT-003-0002)
