

## Protocol for preparation of ColoUp medium

page 1 of 1

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Evercyte Ord. No.:	MHT-039
Reagents:	The ColoUp medium for cultivation of HCEC-1CT cells can either be ordered from Evercyte
	as ready-to-use medium (Cat# MHT-039) or as basal medium (Cat# MHT-039-B) plus
	supplements (Cat# MHT-039-S).
	The medium can also be prepared by mixing the following components:
	DMEM (Gibco, Cat # 61965-026) / M199 (Gibco, Cat# 31150022) (4+1)
	2 % Cosmic Calf Serum (Hyclone, Cat# SH30087, ready-to-use)
	20 ng/ml hEGF (Sigma-Aldrich, Cat# E9644)
	10 μg/ml Insulin (Sigma-Aldrich, Cat# I9278, ready-to-use)
	2 μg/ml Apo-Transferrin (Sigma-Aldrich, Cat# T2036)
	5 nM Sodium-Selenite (Sigma-Aldrich, Cat# S5261)
	1 μg/ml Hydrocortisone (Sigma-Aldrich, Cat# H0396)
	- take one bottle (500 ml) of DMEM and discard 100 ml
	- add 100 ml of M199 and mix properly
	- discard 11.6 ml DMEM/M199 mixture
	- add 10 ml Cosmic Calf Serum (ready-to-use)
	- add 500 μl of hEGF stock (20 μg/ml, prepared in cell culture grade water)
	- add 500 μl of Insulin (10 mg/ml, ready-to-use)
	- add 100 μl of Apo-Transferrin stock (10 mg/ml, prepared in M199)
	- add 25 μl of Sodium-Selenite stock (100 μM, prepared in cell culture grade water)
	- add 500 μl Hydrocortisone stock (1 mg/ml, prepared in cell culture grade water)
	<ul> <li>mix properly and store at 4°C for up to 1 month</li> </ul>
	- temper the medium to room temperature (not 37°C) before use
Related products:	ColoUp ready-to-use medium, 500 ml (Evercyte, Cat#MHT-039)
	ColoUp basal medium, 500 ml (Evercyte, Cat# MHT-039-B)
	ColoUp supplements (Evercyte, Cat# MHT-039-S)
	HCEC-1CT, human colon epithelial cells (Evercyte, Cat# CkHT-039-0229)