

## Protocol for preparation of ColoUp medium

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Evercyte Ord. No.:	MHT-039
Designation:	HCEC-1CT, human colon epithelial cells
Medium components and preparation:	ColoUp medium contains the herein described components and is prepared as described in the following:
	DMEM (Gibco, Cat # 61965-026) / M199 (Gibco, Cat# 31150022) (4+1)
	2 % Cosmic Calf Serum (e.g. 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)
	- mix properly and store at 4°C for up to 1 month
	- 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)
	- ColoUp2 (Cat# MHT-039-2, for US customers), this medium contains all components
	of ColoUp but Cosmic Calf Serum; before use, 10 ml Cosmic Calf Serum must be
	added to give rise to ready-to-use ColoUp medium