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biogems
A PeproTech Brand

Facilitate your research on

Gastrointestinal Epithelium Cells with Organoid Cultures

The *in vitro* studies of gastrointestinal (stomach, small intestinal and colonic) epithelium has long been hampered by the lack of suitable culture systems. Although a variety of organ/organoid and epithelial cell culture models have been developed, most are restricted to short-term application due to the rapid apoptosis that ails intestinal cells once they have been removed from the basement membrane and underlying stroma

The ability to source normal epithelial cell lines from the stem cells found in the base of intestinal crypts has allowed for the establishment of long-term systems for intestinal epithelium cultures. This advancement has allowed for the exploitation of stem cells in tissue regenerative therapies, and the development of treatment models targeting degenerative disorders of the digestive tract.

The model for a robust, long-term small intestinal epithelium organoid culture system was developed in 2009 [1]. Lgr5+ stem cells isolated from murine crypts were cultured with ROCK inhibitor (**Y-27632**) and the ENR growth factor bundle of **EGF (Epidermal Growth Factor), Noggin, and R-Spondin-1**. This culture system mimicks normal intestinal epithelial growth and differentiation, and is able to maintain these characteristics for more than eight months.

Subsequently, a protocol for long-term organoid culturing of human small intestinal epithelium, and both murine and human colonic epitheliums was established. This system added the necessary signaling protein **Wnt-3A** to the above mentioned ENR growth factor bundle (WENR).

In the case of human small intestinal and colonic crypt cultures, the further addition of p38 MAPK Inhibitor (**SB 202190**) and TGF-β inhibitor (**A 83-01**) was required [2]. Currently, these protocols are routinely used in studies involving human or mouse intestinal crypt cultures [3, 4].

The addition of **FGF-10 (Fibroblast Growth Factor-10)** to the WENR bundle (WENRF) allowed researchers to establish long-term gastric gland and human small intestinal epithelium cultures [5,6,7,8].

Small molecules can also be an integral component of intestinal cultures, either as a means of directing culture fate or a tool for for studying signaling pathways. The GSK3β inhibitor **CHIR99021** and the Wnt production inhibitor **IWP-2** were employed in the study of how Wnt/β-catenin signaling influences the differentiation of paneth cells [11].

The development of a new primary tissue model based on gut organoids has allowed for the retention of physiological characteristics in culture. These gut organoids expanded from healthy, human small intestinal crypts on Matrigel ECM (Extra Cellular Matrix) with the assistance of the ENR growth factor bundle plus **Wnt-3a, Y-27632, Gastrin, Nicotinamide, A 83-01, SB 202190** and **LY2157299** [12].

PeproTech, along with the PeproTech Brand BioGems, are proud to be able to support your research by providing you with cytokines and small molecules required for gastrointestinal epithelium cell culture protocols.

CYTOKINES USED IN LONG-TERM GASTROINTESTINAL EPITHELIUM CULTURES

Organ	Species	EGF	Noggin	R-Spondin-1	Wnt-3A	FGF-10
Small Intestine	Murine	✓	✓	✓		
	Human	✓	✓	✓	✓	✓*
Colon	Murine	✓	✓	✓	✓	
	Human	✓	✓	✓	✓	
Stomach	Human	✓	✓	✓	✓	✓

✓* = Optional

SMALL MOLECULES AND PEPTIDES USED IN GASTROINTESTINAL CULTURES

Molecule	Description
A 83-01	Potent inhibitor of activin receptor-like kinase (ALK)
CHIR99021	Selective and potent inhibitor of glycogen synthase kinase 3 (GSK-3) and activator of the WNT pathway
Gastrin I	Peptide hormone responsible for regulating gastric acid secretion and promoting gastric mucosal growth
IWP-2	Wnt pathway inhibitor
LY2157299	Potent inhibitor of TGF-β receptor type I (ALK5)
Nicotinamide	Active form of vitamin B3 and a component of the coenzyme NAD
SB 202190	Selective and potent inhibitor of p38 MAP Kinases (MAPK)
Y-27632 Dihydrochloride	Selective inhibitor of Rho-associated coiled-coil forming protein serine/threonine kinase (ROCK) family of protein kinases that selectively competes with ATP to bind to the catalytic site

References:

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