Colon Organoids Kit (Human)

#BFM-001-002

Introduction

According with the new international ethical guidelines, which suggest to reduce the number of *in vivo* experiments, new *in vitro* models that recapitulate the physiology and anatomy of living tissues are strongly requested in medical research. This kit was designed to be upfront with this philosophy. 3D-organ cultures represent the future of biomedical research in which scientists could test their hypotheses in different field of interest including cancer, obesity, neuroscience, cardiovascular disease, environmental risk and nutrition.

Safety Information

Kit contains:
Penicillin 100 units/ml
Streptomycin 100 µg/ml
Gentamicin 40 µg/ml
DMSO 0,2%

Quality Control

Each lot of the kit was tested to ensure consistent product quality.

Instruments

Laminar flow tissue culture hood, incubator at 37°C and 5% CO_2; centrifuges for 2 and 15 ml tubes; pipettes: P1000, P200, P20 and P2; sterile tips; ice; 24 multiwell plate; automatic pipettor.

Reagents

Name	n°	ml	storage	stability
Washing Medium **(WM)**	6	50	4°C	3 months
Growth Medium **(GM)**	10	10	-20°C	3 months
Supplement **(SUP)**	10	1	-20°C	3 months
Recovery Sol 1 **(RS1)**	2	50	4°C	3 months
Recovery Sol 2 **(RS2)**	1	10	4°C	3 months
Optimized Extracellular Matrix **(OEM)**	3	0,5	-20°C	1 Year

For Research Use Only (RUO).

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PROTOCOL

Before starting:

- Thaw OEM overnight in ice and keep in a refrigerator. OEM is soluble in ice and solid from room temperature to 37°C.
- Add 1 ml of SUP to 9 ml of GM and leave at 4°C. Do not refreeze the reconstituted medium.

Thawing Organoids

NB: leave GM at RT for 30' before add to organoids culture (do not pre-warm at 37°C).

- Thaw the cryovial at 37°C in a water bath by gentle agitation.
- Remove the vial from the water bath and decontaminate it by dipping in or spraying with 70% ethanol. Follow strict aseptic conditions in a laminar flow tissue culture hood for all further manipulations.
- Unscrew the cap of the vial and transfer the contents to a sterile centrifuge 15 ml tube containing 9 ml of WM.
- Spin 5' at 0.4 x g (≈ 1000 rpm).
- Remove carefully the supernatant with vacuum leaving 200 µl of medium.
- Add 800 µl of WM and gently resuspend the pellet.
- Transfer in a 1.5 ml tube and centrifuge 5' at 0.4 x g
- Remove the supernatant with a p200 (do not touch the pellet).
- Resuspend the pellet in 15 µl of OEM with a sterile p20 cutted tip.
- Plate a single drop of OEM/organoids mix on the center of a well (24 mw plate).
- Invert the plate and leave in the incubator for 30'.
- Add 500 µl of GM.
- Change the GM every 2 days.
- Check the organoids every day.

Passage for Maintenance

- Carefully remove the medium without touching the organoids.
- Gently wash two times with 500 µl of RS1.
- Add 200 µl of ice cold RS2 with p1000 and breakdown the OEM by pipetting up and down for 10 times.
- Incubate 5' at 37°C in incubator.
- Add 500 µl of ice cold WM and disaggregate organoids by pipetting up and down for 10 times.
- Collect the supernatant and transfer in a 15 ml tube with 9 ml of ice cold WM and leave 5' on ice.
- Centrifuge 5' at 0.4 x g.
- Remove carefully the supernatant with vacuum and leave 200 µl of medium.
- Add 800 µl of WM and gently resuspend the pellet.
- Count the organoids (only aggregate of more than 5 cells).
- Transfer in a sterile 1.5 ml tube, leave 5' on ice and centrifuge 5' at 0.4 x g.
- Plate 1000-3000 organoids in each well.

Ordering Information

Code	Product	Package
BFM-001-002	Colon Organoids Kit (Human)	1 Kit

References

Sato et al., Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009 May 14;459(7244):262-5.

Matano M et al., Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. Nat Med. 2015 Mar;21(3):256-62

Pauli C et al., Personalized in vitro and in vivo cancer models to guide precision medicine. Cancer Discov. 2017 May;7(5):462-477.