Data Sheet

3dGRO™ Human CRC Organoids (ISO48)

Stem Cell Line

Cat. # SCC502 **pack size:** ≥ 100,000 organoids

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Store at: -80ºC

Background

Colorectal cancer accounts for roughly 10% of all cancer cases worldwide with more than half of all patients with CRC developing metastatic disease leading to death. Recently, tissue derived organoids have emerged as a more predictive 3 dimensional cell culture model of disease. 3D organoid cultures conserve the original genetic and phenotypic characteristics of the primary tissue allowing for their application in many research fields included drug development, personalized medicine and potential therapeutics. *In vitro* cultured tumor organoids have also been shown to predict patient repsonse to chemotherapeutics. PDOs derived from colorectal cancer (CRC organoids) have been used for cell modeling and to investigate the function of cancer related driver gene mutations including APC, TP53, KRAS, BRAF, PIK3CA etc.

3dGRO™ Human CRC Organoids utilize Cellesce's patented technology which enables the robust growth and expansion of patient-derived organoids (PDOs). Cellesce's technology minimizes manual handling time to maximise reproducibility in order to position organoid cell models as a cost effective and accurate tool in early-stage drug discovery. The organoid biobank includes tumor derived colorectal cancer PDOs from a range of genetic backgrounds, driver gene mutations, tumor sites and cancer stages. These organoid cell lines have been well characterised and are all validated for response against a number of known CRC-targeting agents.

Storage and Handling

Store 3dGRO™ Human CRC Organoids at -80°C. For long term storage the cells should be stored in liquid nitrogen.

Quality Control Testing

Cell Growth: Organoid expansion after 7 days
Mycoplasma: Negative
Viral Testing: Negative (HIV-1, HIV-2, HBV, CMV, EBV, HPV)
Sterility (Bacteria, Yeast, Fungi): Negative
Mutational Profile: PIK3CA ΔE542K, CTNNB1 ΔS33C

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COLORECTAL CANCER ORGANOID LINE INFORMATION

Characteristic	ISO 34	ISO 38	ISO 48	ISO 49	ISO 50	ISO 57	ISO 68	ISO 72	ISO 75	ISO 78	Characteristic
Tumour Site	Transverse Colon	Sigmoid Colon	Caecum	Lower Sigmoid	Rectum	Upper rectum	Sigmoid Colon	Caecum	Caecum	Caecum	Tumour Site
Dukes' Stage	A	C1	C2	C1	C2	A	C1	B	C1	C1	Dukes' Stage
Media requirement	Media A	Media A	Media A	Media A	Media A	Media A	Media A	Media A	Media B	Media B	Media requirement

Component	Final concentration	Media A	Media B
Advanced DMEM-F12	1X	✓	✓
HEPES	10mM	✓	✓
Glutamax	2mM	✓	✓
Penicillin/Streptomyocin	100 U/ml	✓	✓
B27	1X	✓	✓
N2	1X	✓	✓
N-acetyl-L-cysteine	1.25mM	✓	✓

Component	Final concentration	Media A	Media B
EGF	50 ng/ml		✓
Noggin	100 ng/ml		✓
Nicotinamide	10 mM		✓
A8301	500nM		✓
SB202190	10µM		✓
Wnt Conditioned Media	40%		✓
R-Spondin 1 Conditioned Media	10%		✓

Organoid media compositions: based on line-dependent requirements

MUTATIONAL PROFILE

Gene	ISO 34	ISO 38	ISO 48	ISO 49	ISO 50	ISO 57	ISO 68	ISO 72	ISO 75	ISO 78	Gene
APC	▲ E1451*	▲ G1339Ffs*2	---	▲ R1450* A1446Lfs*27	▲ R232* E1286*	▲ Q978*	▲ Q1096* E1408*	▲ Q1291*	---	▲ R876* E1451*	APC
TP53	---	▲ C238Y	---	▲ R248Q	▲ R248Q"	▲ R282W	▲ R248W	---	---	▲ H193D	TP53
KRAS	---	---	---	▲ G12D	▲ G12D	▲ G13D	▲ G13D	▲ G12D	---	▲ G12D	KRAS
BRAF	▲ K601E	---	---	---	---	---	---	---	▲ V600E	---	BRAF
PIK3CA	---	---	▲ E542K	▲ E542K	---	---	---	---	---	---	PIK3CA
CTNNB1	---	---	▲ S33C	---	---	---	---	---	---	---	CTNNB1
FBXW7	---	---	---	▲ R465C	---	---	---	---	---	---	FBXW7
ARID1A	---	---	---	---	---	---	---	---	▲ F2141Sfs*59	---	ARID1A
SMAD4	---	▲ D537H	---	---	▲ E526K	---	▲ Q534*	---	---	---	SMAD4
ARID2	---	---	---	---	---	---	---	---	---	---	ARID2
AXIN2	---	---	---	---	---	---	---	---	---	---	AXIN2
ERBB3	---	---	---	---	---	---	---	▲ A232V	---	---	ERBB3
MSH3	---	---	---	---	---	---	---	---	▲ K381Gfs*20	---	MSH3
NRAS	---	---	---	---	---	---	---	---	---	---	NRAS
POLE	---	---	---	---	---	---	---	---	---	---	POLE
SMAD2	---	---	---	---	▲ S464*	---	---	---	---	---	SMAD2
TCF7L2	---	---	---	---	---	---	---	---	---	---	TCF7L2
RNF43	---	---	---	---	---	---	---	---	▲ G659GX	---	RNF43
Gene	ISO 34	ISO 38	ISO 48	ISO 49	ISO 50	ISO 57	ISO 68	ISO 72	ISO 75	ISO 78	Gene

Figure 1. 3dGRO™ Human CRC Organoid cell line info and mutational profiles. *Note: Full list of 1608 genes screened available upon request.*

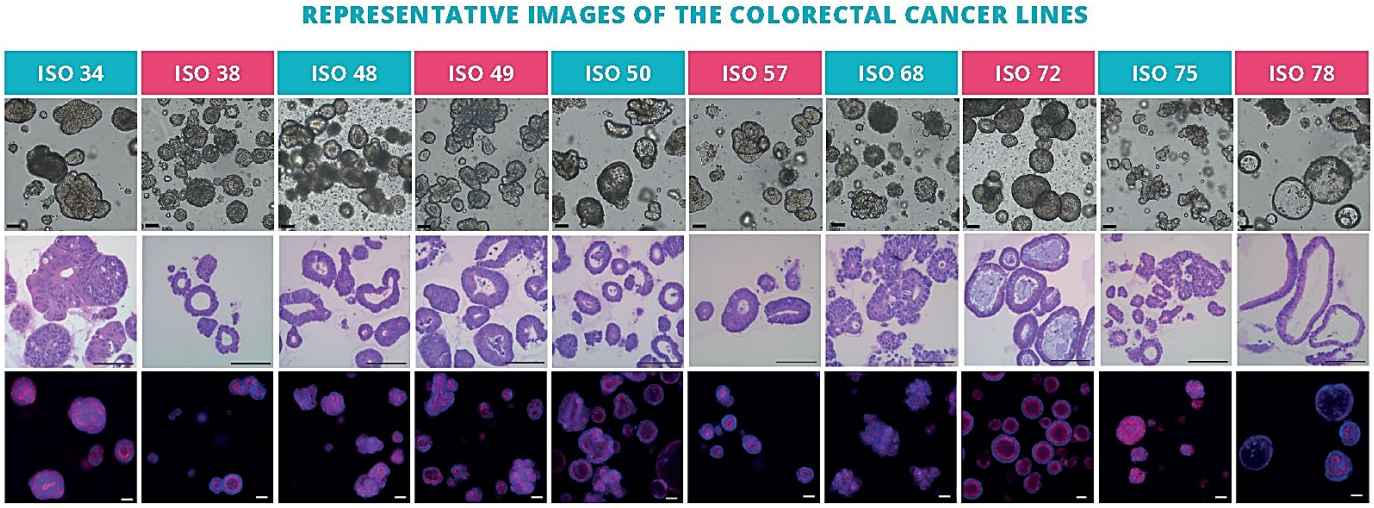

Figure 2. Morphology of colorectal cancer organoids. Brightfield, H&E, and confocal images of CRC organoids. Bottom: organoids stained for nuclear (blue) and cytoskeletal (red) markers for confocal imaging. Scale 100µm.

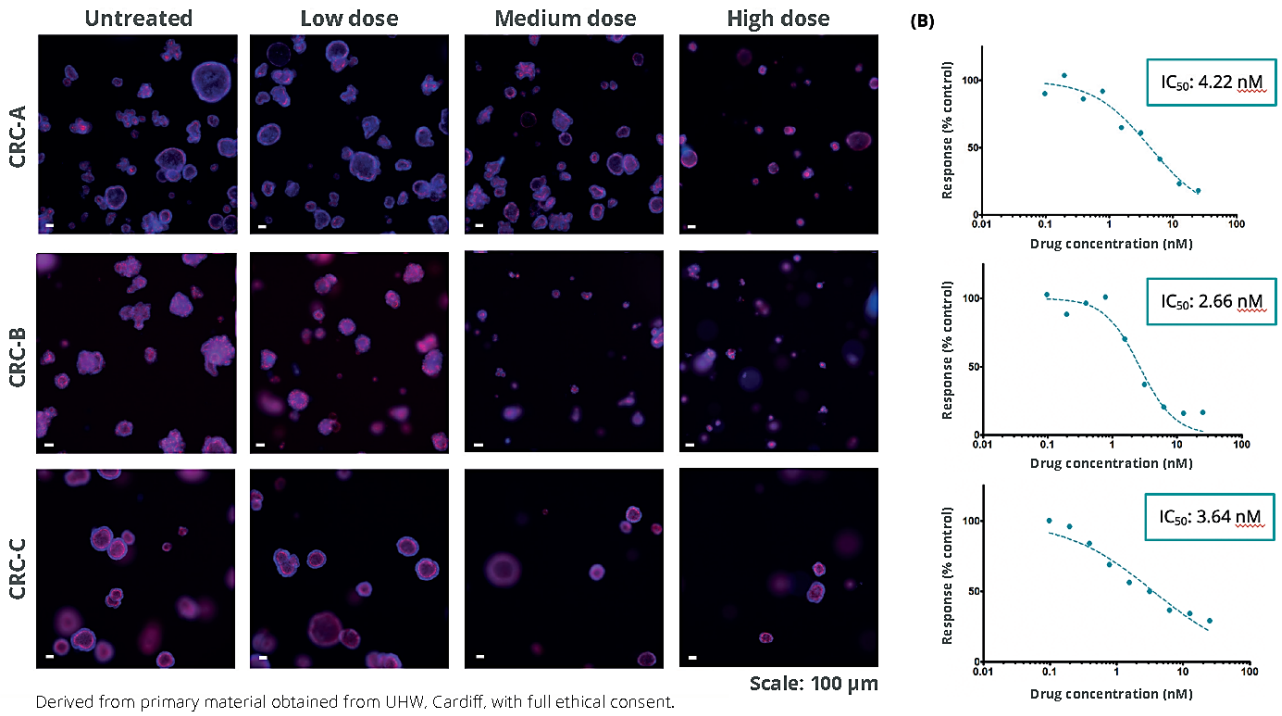

Figure 3. Drug screening using 3dGRO™ Human CRC Organoids. CRC organoids were thawed and allowed to recover for 48 hours followed by titration of Trametinib alongside a DMSO control over 5 days. Organoids were then stained for nuclear (blue) and cytoskeletal (red) markers for confocal imaging. Assay end point organoid viability was determined by CellTiterGlo 3D, to generate titration curves and IC_{50} values. Scale bars: 100 µm. *CRC-A: ISO50,CRC-B: ISO68, CRC-C: ISO72.*

Protocols

All protocols are performed within a Class II laminar flow biohood and with an aspirator unless otherwise specified. Incubators are humidified and are set to 37°C and 5% CO_2. PPE should be worn – gloves, lab coat and safety glasses.

Additional Material Required

- DMEM/F-12 PLUS Basal Medium: **Sigma (SCM162)**
- Glutamax (100X): **Invitrogen (35050-079)**
- HEPES (1M): **Sigma (H0887)**
- Penicillin/Streptomycin (100X): **Sigma (P4333)**
- B27 (50x): **Invitrogen (17504-044)**
- N2 (100x): **Invitrogen (17502-048)**
- N-Acetylcysteine (500 mM): **Sigma (A9165-5G)**
- Growth factor reduced Matrigel: **Corning (356231)**
- Rho Kinase Inhibitor (Y27632) (10mM): **Millipore (688002)**
- Bovine Serum Albumin: **Sigma (A9418)**
- PBS: **Sigma (D8537)**

Preparation of Base Media

1. To prepare 500 mL of base media, add 5ml HEPES, 5ml Glutamax and 5ml Penicillin/Streptomycin to a 500ml bottle of DMEM/F12 Plus Basal Media.
2. Mix well. Store base media at 4°C for no longer than 4 weeks.

Preparation of Complete Media

1. Follow media guidelines in figure 1. To prepare 50 mL of complete media, take 48.38 mL of Advanced DMEM/F12 Plus Basal Media from above and add: 0.5 mL N2, 1 mL B27, 0.125 mL N-acetyl cysteine.
2. Mix well. Store complete media at 4°C for no longer than 2 weeks.

Thawing of Organoids

This protocol gives details of suggested plating for a 96 well plate (triplicate determinations), with adjustments given for 384 well plating (quadruplicate determinations). Well numbers are provided assuming a 9 point drug titration plus control wells.

1. For each vial you will be thawing/plating, add 10 mL prewarmed base media to a 15 mL tube (precoated in 1% BSA) and label.
2. Take each vial you will use from the -80°C freezer or cryotank and thaw quickly in 37°C waterbath, until a small amount remains frozen (this will thaw by the time of the next step).
3. Add 0.8 mL pre-warmed base media to the vial, mix gently with a P1000 tip and transfer the contents of the vial (1 mL) to its corresponding tube.
4. Wash the vial with a further 1 mL base media and add this to the tube.
5. Make up the volume in the tube to 10 mL and centrifuge the contents for 5 minutes at 100xg.
6. Aspirate to 1 mL, mix gently with a P1000 tip then resuspend to 10 mL with base media. Your organoids will now be resuspended at a density of 10,000 organoids/mL.
7. Calculate the number of organoids required for your assay type, with an added contingency to allow for Matrigel/pipetting error.

8. Mix the organoid suspension gently but thoroughly, and pipette the required volume of organoid suspension into a labelled, fresh 15 mL tube (pre-coated with 1% BSA) and resuspend each to 10 mL with base media to wash.
9. Centrifuge the contents of the tubes for 5 minutes at 200 xg.
10. Aspirate the supernatant away, leaving a small volume (up to 50 µL) media in each tube. Keep the tubes on ice. Set a fresh 1.6 mL microcentrifuge tube on ice, also labelled appropriately.
11. For ease of plating, organoids should be resuspended in 80% Matrigel, with the remaining 20% volume made up with base media. This reduces the viscosity of the Matrigel and can aid consistency of plating. Calculate the volume of base media and Matrigel required to plate your organoids.
12. To resuspend, begin by adding a small volume of the base media required and pipetting slowly to avoid bubbles, and keeping all tubes on ice. Once, thoroughly resuspended, add the remaining volume of base media and mix well.
13. Then add in the required volume of Matrigel and ensure thorough (but gentle) resuspension of the mixture.
14. Plate out the suspension: dispense 10 µL of Matrigel/organoid suspension into each well of a 96 well plate. The droplet should form a dome shape at the bottom of each well.
15. Ensure thorough mixing of the Matrigel/organoid suspension regularly throughout plating as organoids may sink. It is suggested to leave the edge wells of the plate empty.
16. Allow Matrigel to solidify by incubating the plate at 37°C for 10 minutes.
17. Following incubation, add 50 µL of complete media, supplemented with 10 µM ROCK inhibitor (i.e. add 1µL of ROCK inhibitor stock solution per mL of complete media), to each well containing organoids.
18. Add 50 µL of DMEM/F12 PLUS basal medium or PBS to each edge well.
19. Incubate plates at 37°C, 5%$C0_2$.
20. Replace the media with complete media (no ROCK inhibitor) after 2 days (or compounds of your choice, diluted to your desired concentrations in complete media).
21. Replace media/compounds as often as required for your individual assay.