

Subculture and Cryopreservation of Esophageal Adenocarcinoma Organoids: Pros and Cons for Single Cell Digestion

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Materials

Name	Company	Catalog Number	Comments
Equipment			
-20°C Freezer	Bosch		Economic
-80°C Freezer	Panasonic		MDF DU500VH-PE
Automated Cell counter	Thermo Fisher	AMQAX1000	Countess II
Biological Safety Cabinet Class II	Thermo Scientific	51022482	Herasafe KS12
Centrifuge	Heraeus	75003060	Megafuge 1.0R
CO ₂ Incubator	Thermo Scientific	50116048	Heracell 150i
Inverted automated fluorescence microscope	Olympus		IX83
Inverted light microscope	Leica		DMIL LED Fluo
Pipette 1000 µL	Eppendorf	3123000063	Research Plus
Pipette 200 µL	Eppendorf	3123000039	Research Plus
Rotating Incubator	Scientific Industries, sc.	SI-1200	Enviro-genie
Shaker	Eppendorf	5355 000.011	Thermomixer Comfort
Vacuum pump	Vacuubrand	20727200	BVC control
Waterbath	Medingen	p2725	W22
Material			
15 mL tube	Sarstedt	62.554.502	Inc Screw cap tube PP 15 mL
Cryo vial 2 mL	Sarstedt	72.379	CryoPure 2.0 mL tube
Low bind tube 1.5 mL	Sarstedt	72.706.600	Micro tube 1.5 mL protein LB
Low bind tube 5 mL	Eppendorf	0030 108.302	Protein LoBind Tube 5.0 mL
Pipette tip 200 µL	Starlab	E1011-8000	200 µL Graduated tip, wide orifice
Pipette tip 1000 µL	Starlab	E1011-9000	1000 µL Graduated tip, wide orifice
Pipette tip 1000 µL	Sarstedt	70.3050	Pipette tip 1000 µL
Sterile filter 0.2 µm	Sarstedt	83.1826.001	Filtropur 0.2 µm sterile filter

Tissue culture plate	Sarstedt	83.3921	12 well-plate
Reagent/Chemical			
A83-01	Tocris	2939	
Advanced DMEM/F-12	Thermo Fisher Scientific	12634010	
Amphotericin B	Thermo Fisher Scientific	15290026	
B-27	Thermo Fisher Scientific	17504001	
Cell Recovery Solution	Corning	354253	
CHIR-99021	MedChemExpress	HY-10182/CS-0181	
DNase I grade II, from bovine pancreas	Sigma-Aldrich	10104159001	
Dulbecco's phosphate-buffered saline (DPBS)	Thermo Fisher Scientific	14190094	
Extracellular matrix (ECM) gel: Matrigel Growth Factor Reduced (GFR) Basement Membrane Matrix	Corning	356231	
FGF-10a	Peprtech	100-26-100	
Freezing medium: Recovery Cell Freezing Medium	Thermo Fisher Scientific	12648010	
Gastrin	Sigma	G9020	
Gentamicin-25 (25 mg/ 500 µL)	PromoCell	C-36030	
HEPES (1 M)	Thermo Fisher Scientific	15630080	
L-Glutamine 200 mM (100X)	Thermo Fisher Scientific	25030024	
N-2	Thermo Fisher Scientific	17502-048	
N-Acetylcysteine	Sigma	A9165	
Nicotinamide	Sigma	N0636-100	
Noggin	Peprtech	120-10C-50	
Penicillin-Streptomycin 10,000 U/ mL (100X)	Thermo Fisher Scientific	15140122	
Recombinant human epidermal growth factor (EGF)	Peprtech	AF-100-15	
R-Spondin1 conditioned medium from Cultrex R-Spondin Cells	Biotechne	3710-001-01	
SB202190	MedChemExpress	152121-30-7	
Trypsin inhibitor from Glycine max (soybean)	Sigma-Aldrich	93620-1G	
Trypsin-EDTA (0.25 %), phenol red	Thermo Fisher Scientific	25200056	
Wnt-3A conditioned medium	Wnt-3A expressing cell line was kindly provided by Prof. Hans Clevers' group		
Y-27632	Sigma	Y0503	