

SOP30101: Recipes for Complete Media for Patient-Derived In Vitro and Organoid Cultures

Effective Date: 1/9/2024

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PDMR NCI Patient-Derived Models Repository
An NCI Precision Oncology InitiativeSM Resource

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VERSION INFORMATION

1. Change History

Revision	Description
	Internal SOP used by PDMR In Vitro Laboratory
10/15/2017	Standardize SOP for posting to PDMR internal site for use by designated NCI intramural laboratories
5/14/2018	Updated reference SOPs and Purpose/Scope section
7/2/2018	Merged PDC/CAF and PDOrg media SOPs. Added explanation of organoid-derived PDCs using organoid media + 10% FBS.
9/13/2018	Updated Y-compound preparation to use sterile water. Added Breast #2 Culture Media recipe
1/16/2019	Added alternate commercial resource for several reagents.
12/4/2020	Updated Hydrocortisone and Adenine stock solution preparations for Complete DMEM/F12 Media
1/9/2024	Added Contributor media recipe for Cholangiocarcinoma PDCs

2. Related SOPs

SOP30102: Preparation of Matrigel-Coated Flasks for Adherent Patient-Derived In Vitro Cultures
SOP30103: Initial Culture, Sub-culture, and Cryopreservation of Adherent Patient-Derived Tumor Cultures (PDCs)
SOP30104: Initial Culture, Sub-culture, and Cryopreservation of Suspension Patient-Derived Tumor Cultures (PDCs)
SOP30105: Initial Culture and Sub-culture of Patient-Derived Cancer-Associated Fibroblasts (CAFs)
SOP40102: Thawing and Initial Culture of Patient-Derived Organoid (PDOrg) Cultures
SOP40103: Passaging and Sub-culture of Patient-Derived Organoid (PDOrg) Cultures
SOP40104: Cryopreservation of Patient-Derived Organoid (PDOrg) Cultures

1.0 PURPOSE/SCOPE

This Standing Operating Procedure (SOP) describes common tissue culture media used for successful recovery of Patient-Derived Tumor Cultures (PDCs), Cancer-Associated Fibroblasts (CAFs), and Organoids (PDOrg) from cryopreservation and sub-culture under BSL-2 safety criteria. Early-passage patient-derived in vitro cultures require different growth conditions, have different growth characteristics, and visually appear different than traditional cell cultures. The recommended tissue culture media for **each specific** culture are provided as part of the Certificate of Analysis for the culture. **Not all cultures will use the same media.**

This SOP is used/performed by the Biological Testing Branch (BTB) at NCI-Frederick, Frederick National Laboratory for Cancer Research.

2.0 SAFETY

BTB treats all patient-derived in vitro cell cultures under Biosafety Level 2 (BSL2) conditions even when PCR-based screening has not detected the presence of a known set of human pathogens. All work is conducted in a biological safety cabinet (BSC) using personal protective equipment and avoiding the use of sharps where possible. All materials potentially exposed to the cell cultures are disinfected by exposure to a 10% bleach solution for a minimum of 10 minutes, double bagging for autoclaving or incineration. Consult with your facility safety professionals regarding the safe handling of BSL2 studies.

3.0 CLEAN-UP

- 3.1 All materials in contact with patient tissue, as well as the mice carrying patient tumor samples and cultures derived from patient tumor samples, are treated as a potential health threat (BSL-2 precautions) since the human tissues could retain human pathogenic agents even if they do not replicate in mouse cells (e.g., EBV, HPV, etc).
- 3.2 Flush/soak any items (e.g., tubes, syringes, petri dishes, lab mats, etc) that were in contact with human tissue with disinfectant (e.g., 10% bleach, commercial hydrogen peroxide disinfectant, 2% Virkon®) for a minimum of 10 minutes before disposal in biohazard waste or sharps containers (follow institutional guidelines and manufacturer's recommendations).
- 3.3 For items that can't be rinsed (e.g., micropipettors), wipe down thoroughly with bleach-soaked gauze or other appropriate disinfectants.

4.0 EQUIPMENT

4.1 Reagents for Stock Solution Preparations

- 4.1.1** UltraPure DNase/RNase-free distilled water (e.g., Quality Biological, Cat#: 118-162-131)
- 4.1.2** DPBS, no calcium, no magnesium (Thermo Fisher Scientific, Cat#: 14190250)
- 4.1.3** DMSO, HPLC-grade, >99.5% pure (Honeywell Research Chemicals/Burdick & Jackson, Cat#: 081 1L)
- 4.1.4** Bovine Serum Albumin (BSA; Sigma, Cat#: A-4503)
- 4.1.5** Ethanol, 200 proof, >99.5% purity (e.g., Pharmco-AAPER, Cat#: 111000200)
- 4.1.6** Hydrochloric acid, HCl (e.g., Sigma Aldrich Cat#: 320331-500mL)

4.2 Material & Equipment

- 4.2.1** 50-mL, 25-mL, 10-mL, 5-mL pipettes, sterile
- 4.2.2** Pipetman and sterile tips
- 4.2.3** 0.22 µm, Sterile Filter Unit, 500 mL
- 4.2.4** Waste container Bleach (Clorox, 5.25% Hypochlorite) diluted 1:10, 2% Virkon®, or similar disinfectant
- 4.2.5** Refrigerator (4°C) and freezer (−20°C)
- 4.2.6** Biological Safety Cabinet (BSC) meeting biosafety level 2 (BSL2) standards
- 4.2.7** Personal Protective Equipment (PPE) at a minimum laboratory coat, with fitted sleeves, latex or nitrile gloves and safety glasses

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5.0 COMPLETE DMEM/F12 MEDIA (FINAL VOLUME 500 ML)

Primary base media for in vitro PDC and CAF cultures. Review Certificate of Analysis for each culture as some organoid-derived PDC cultures require use of the organoid-specific media + 10% FBS.

5.1 Reagents for Complete DMEM/F12 Media

Item	Catalog
Advanced DMEM/F12 1X	Invitrogen, Cat#: 12634-010
Fetal Bovine Serum	Hyclone, Cat#: SH30070.03HI or SH30071.03HI
Hydrocortisone	Sigma, Cat#: H4001
EGF Recombinant Human Protein	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236
Adenine	Sigma, Cat#: A2786
Pen/Strep, 10000 U/mL or Primocin, 50 mg/mL	Invitrogen, Cat#: 1514022 InvivoGen, Cat#: ant-pm-2
L-Glutamine, 200 mM	Invitrogen, Cat#: 25030-081
Y-27632 dihydrochloride*	Tocris Bioscience: Cat# 1254

5.2 Prepare Complete Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock solution	Volume	Working Concentration
Advanced DMEM/F12 1X	--	459-463 mL	
Fetal Bovine Serum	100%	25 mL	5%
Hydrocortisone	1 mg/mL in 20% EtOH (ice-cold/ultrapure water)	200 µL	0.4 µg/mL
EGF Recombinant Human Protein	0.2 mg/mL in DPBS, prepared according to manufacturer's instructions	25 µL	0.01 µg/mL
Adenine	2.4 mg/mL in 50 mM HCl	5 mL	24 µg/mL
Pen/Strep or Primocin	10000 U/mL 50 mg/mL	5 mL or 1 mL	100 U/mL 0.1 mg/mL
L-Glutamine	200 mM	5 mL	2mM
Y-27632 dihydrochloride*	10 mM in Sterile Water (high grade) (3.2 mg/mL)	500 µL	10 µM

*Note: Y compound is included upon thawing of cultures and until culture is established (see SOP30103, SOP30104, or SOP30105). Growth of cells in absence of Y-compound is cell dependent and is noted in the individual Certificate of Analysis.

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6.0 PDORG BASIC MEDIA

Used as the base media for all PDOrg Complete Feeding Medias

NOTE: PDC cultures (2D; grown on plastic/coated surface) derived from organoids sometimes require a PDOrg media. For these cultures, add 10% FBS to the final Basic Media recipe.

6.1 Reagents; follow manufacturer's recommendations

Item	Catalog
Advanced DMEM/F12 (1X)	Invitrogen, Cat#: 12634-028
HEPES (1M)	Invitrogen, Cat#: 15630080
GlutaMax Supplement (100X)	Life Technologies, Cat#: 35050061
Primocin (50 mg/mL)	InvivoGen, Cat#: Ant-pm-2

6.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
Advanced DMEM/F12		500mL	
HEPES	1M	5 mL	10 mM
GlutaMax Supplement	100X	5 mL	1X
Primocin	50mg/mL	1 mL	0.1 mg/mL

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7.0 PDORG COMPLETE FEEDING MEDIA RECIPES

7.1 Media Type: 6A (Final Volume 500 mL)

7.1.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
L-WRN Conditioned Media	Details in SOP Section 9.0
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254

7.1.1 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDORG Basic Media		250 mL	
L-WRN Conditioned Media	100%	250 mL	50%
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	1.25 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	5 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	10 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	5 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	500 µL	10 µM

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7.2 Media Type: 6B/Colon 1A (Final Volume 500 mL)

7.2.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254

7.2.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		500 mL	
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	1.25 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	5 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	10 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	5 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	500 µL	10 µM

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7.3 Media Type: 6C/Colon 1B (Final Volume 500 mL)

7.3.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
L-WRN Conditioned Media	Details in SOP Section 9.0
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236

7.3.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		250 mL	
L-WRN Conditioned Media	100%	250 mL	50%
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	1.25 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	5 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	10 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	5 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	500 µL	10 µM
hEGF	50 µg/mL in DPBS	500 µL	50 ng/mL

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7.4 Media Type: 6D (Final Volume 500 mL)

7.4.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236

7.4.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		500 mL	
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	1.25 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	5 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	10 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	5 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	500 µL	10 µM
hEGF	50 µg/mL in DPBS	500 µL	50 ng/mL

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7.5 Media Type: 6E (Final Volume 500 mL)

7.5.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
L-WRN Conditioned Media	Details in SOP Section 9.0
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236
Recombinant Human FGF-10 (hFGF-10)	PeproTech, Cat#: 100-26; R&D Systems Cat # 345-FG
Recombinant Human FGF-basic (hFGF-2)	PeproTech, Cat#: 100-18B; R&D Systems, Cat#: 233-FB
Prostaglandin E ₂ (PGE ₂)	Tocris, Cat#: 2296; R&D Systems, Cat#: 2296

7.5.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		250 mL	
L-WRN Conditioned Media	100%	250 mL	50%
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	1.25 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	5 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	10 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	5 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	500 µL	10 µM
hEGF	50 µg/mL in DPBS	500 µL	50 ng/mL
hFGF-10	25 µg/250 µL 0.1% BSA in DPBS	50 µL	10 ng/mL
hFGF-2	10 µg/mL in 0.1% BSA	50 µL	1 ng/mL
PGE ₂	20 mM (10 mg/1.42 mL) in DMSO	25 µL	1 µM

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7.6 Media Type: 6F (Final Volume 500 mL)

7.6.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236
Recombinant Human FGF-10 (hFGF-10)	PeproTech, Cat#: 100-26; R&D Systems Cat # 345-FG
Recombinant Human FGF-basic (hFGF-2)	PeproTech, Cat#: 100-18B; R&D Systems, Cat#: 233-FB
Prostaglandin E ₂ (PGE2)	Tocris, Cat#: 2296; R&D Systems, Cat#: 2296

7.6.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		500 mL	
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	1.25 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	5 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	10 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	5 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	500 µL	10 µM
hEGF	50 µg/mL in DPBS	500 µL	50 ng/mL
hFGF-10	25 µg/250 µL 0.1% BSA in DPBS	50 µL	10 ng/mL
hFGF-2	10 µg/mL in 0.1% BSA	50 µL	1 ng/mL
PGE2	20 mM (10 mg/1.42 mL) in DMSO	25 µL	1 µM

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7.7 Media Type: 6G (Final Volume 500 mL)

7.7.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
L-WRN Conditioned Media	Details in SOP Section 9.0
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236
Recombinant Human FGF-10 (hFGF-10)	PeproTech, Cat#: 100-26; R&D Systems Cat # 345-FG
Recombinant Human FGF-basic (hFGF-2)	PeproTech, Cat#: 100-18B; R&D Systems, Cat#: 233-FB
Prostaglandin E ₂ (PGE ₂)	Tocris, Cat#: 2296; R&D Systems, Cat#: 2296
SB-431542	Selleckchem, Cat#: S1067; R&D Systems, Cat#: 1614
SB-202190	Sigma, Cat#: S7067-5MG; R&D Systems, Cat#: 1264

7.7.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		250 mL	
L-WRN Conditioned Media	100%	250 mL	50%
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	1.25 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	5 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	10 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	5 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	500 µL	10 µM
hEGF	50 µg/mL in DPBS	500 µL	50 ng/mL
hFGF-10	25 µg/250 µL 0.1% BSA in DPBS	50 µL	10 ng/mL
hFGF-2	10 µg/mL in 0.1% BSA	50 µL	1 ng/mL
PGE ₂	20 mM (10 mg/1.42 mL) in DMSO	25 µL	1 µM
SB-431542	10 mM in DMSO	25 µL	500 nM
SB-202190	10 mM (5 mg/1.5 mL DMSO)	500 µL	10 µM

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7.8 Media Type: 6H (Final Volume 300 mL)

7.8.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254; R&D Systems, Cat#: AFL236
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236
Recombinant Human FGF-10 (hFGF-10)	PeproTech, Cat#: 100-26; R&D Systems, Cat#: 345-FG
Recombinant Human FGF-basic (hFGF-2)	PeproTech, Cat#: 100-18B; R&D Systems, Cat#: 233-FB
Prostaglandin E ₂ (PGE ₂)	Tocris, Cat#: 2296; R&D Systems, Cat#: 2296
SB-431542	Selleckchem, Cat#: S1067; R&D Systems, Cat#: 1614
SB-202190	Sigma, Cat#: S7067-5MG; R&D Systems, Cat#: 1264

7.8.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		300 mL	
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	0.75 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	3 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	6 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	3 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	300 µL	10 µM
hEGF	50 µg/mL in DPBS	300 µL	50 ng/mL
hFGF-10	25 µg/250 µL 0.1% BSA in DPBS	30 µL	10 ng/mL
hFGF-2	10 µg/mL in 0.1% BSA	30 µL	1 ng/mL
PGE ₂	20 mM (10 mg/1.42 mL) in DMSO	15 µL	1 µM
SB-431542	10 mM in DMSO	15 µL	500 nM
SB-202190	10 mM (5 mg/1.5 mL DMSO)	300 µL	10 µM

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7.9 Media Type: 6I (Final Volume 300 mL)

7.9.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
L-WRN Conditioned Media	Details in SOP Section 9.0
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236
Recombinant Human FGF-10 (hFGF-10)	PeproTech, Cat#: 100-26; R&D Systems, Cat#: 345-FG
Recombinant Human FGF-basic (hFGF-2)	PeproTech, Cat#: 100-18B; R&D Systems, Cat#: 233-FB
Prostaglandin E ₂ (PGE ₂)	Tocris, Cat#: 2296; R&D Systems, Cat#: 2296
Hydrocortisone	Sigma, Cat#: H4001-1G
Insulin (Bovine)	Gemini Bio-Products, Cat#: 700-112P

7.9.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		150 mL	
L-WRN Conditioned Media	100%	150 mL	50%
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	0.75 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	3 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	6 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	3 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	300 µL	10 µM
hEGF	50 µg/mL in DPBS	300 µL	50 ng/mL
hFGF-10	25 µg/250 µL 0.1% BSA in DPBS	30 µL	10 ng/mL
hFGF-2	10 µg/mL in 0.1% BSA	30 µL	1 ng/mL
PGE ₂	20 mM (10 mg/1.42 mL) in DMSO	15 µL	1 µM
Hydrocortisone	1 mg/mL in 10% EtOH/ultrapure water	90 µL	0.3 µg/mL
Insulin (Bovine)	2 mg/mL in 0.1 M HCl	150 µL	1 µg/mL

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7.10 Media Type: 6J (Final Volume 300 mL)

7.10.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
L-WRN Conditioned Media	Details in SOP Section 9.0
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236
Recombinant Human FGF-10 (hFGF-10)	PeproTech, Cat#: 100-26; R&D Systems, Cat#: 345-FG
Recombinant Human FGF-basic (hFGF-2)	PeproTech, Cat#: 100-18B; R&D Systems, Cat#: 233-FB
Prostaglandin E ₂ (PGE ₂)	Tocris, Cat#: 2296; R&D Systems, Cat#: 2296
SB-431542	Selleckchem, Cat#: S1067; R&D Systems, Cat#: 1614
SB-202190	Sigma, Cat#: S7067-5MG; R&D Systems, Cat#: 1264
Hydrocortisone	Sigma, Cat#: H4001-1G
Insulin (Bovine)	Gemini Bio-Products, Cat#: 700-112P

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7.10.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		150 mL	
L-WRN Conditioned Media	100%	150 mL	50%
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	0.75 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	3 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	6 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	3 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	300 µL	10 µM
hEGF	50 µg/mL in DPBS	300 µL	50 ng/mL
hFGF-10	25 µg/250 µL 0.1% BSA in DPBS	30 µL	10 ng/mL
hFGF-2	10 µg/mL in 0.1% BSA	30 µL	1 ng/mL
PGE2	20 mM (10 mg/1.42 mL) in DMSO	15 µL	1 µM
SB-431542	10 mM in DMSO	15 µL	500 nM
SB-202190	10 mM (5 mg/1.5 mL DMSO)	300 µL	10 µM
Hydrocortisone	1 mg/mL in 10% EtOH/ultrapure water	90 µL	0.3 µg/mL
Insulin (Bovine)	2 mg/mL in 0.1 M HCl	150 µL	1 µg/mL

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7.11 Media Type: Breast #1 (Final Volume 500 mL)

7.11.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
L-WRN Conditioned Media	Details in SOP Section 9.0
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236
Recombinant Human FGF-10 (hFGF-10)	PeproTech, Cat#: 100-26; R&D Systems Cat # 345-FG
Recombinant Human FGF-basic (hFGF-2)	PeproTech, Cat#: 100-18B; R&D Systems, Cat#: 233-FB
Hydrocortisone	Sigma, Cat#: H4001-1G
Insulin (Bovine)	Gemini Bio-Products, Cat#: 700-112P
β-estradiol	Sigma, Cat#: E2758-1G

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7.11.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		250 mL	
L-WRN Conditioned Media	100%	250 mL	50%
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	1.25 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	5 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	10 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	5 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	500 µL	10 µM
hEGF	50 µg/mL in DPBS	100 µL	10 ng/mL
hFGF-10	25 µg/250 µL 0.1% BSA in DPBS	50 µL	10 ng/mL
hFGF-2	10 µg/mL in 0.1% BSA	50 µL	1 ng/mL
Hydrocortisone	1 mg/mL in 10% EtOH/ultrapure water	150 µL	0.3 µg/mL
Insulin	2 mg/mL in 0.1 M HCl	250 µL	1 µg/mL
β-estradiol*	2 mM Stock solution in 100% EtOH*; 20 µM Working Stock solution in PDOrg Basic Media	12.5 µL	0.5 nM

*β-estradiol stock and working stock preparation recommendations to ensure stability and to maintain reagent in solution. Prepare 2 mM Stock solution in 100% EtOH for long-term storage per manufacturer's recommendations. Just before use, make a Working Stock solution by diluting 1:10 twice to keep volume low and ensure pipetting accuracy (1:100 total) using PDOrg Basic Media for a Working stock concentration of 20 µM. Use Working Stock solution to prepare Media.

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7.12 Media Type: Breast #2 (Final Volume 500 mL)

7.12.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
L-WRN Conditioned Media	Details in SOP Section 9.0
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236
Recombinant Human FGF-10 (hFGF-10)	PeproTech, Cat#: 100-26; R&D Systems Cat # 345-FG
Recombinant Human FGF-basic (hFGF-2)	PeproTech, Cat#: 100-18B; R&D Systems, Cat#: 233-FB

7.12.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		250 mL	
L-WRN Conditioned Media	100%	250 mL	50%
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	1.25 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	5 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	10 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	5 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	500 µL	10 µM
hEGF	50 µg/mL in DPBS	100 µL	10 ng/mL
hFGF-10	25 µg/250 µL 0.1% BSA in DPBS	50 µL	10 ng/mL
hFGF-2	10 µg/mL in 0.1% BSA	50 µL	1 ng/mL

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7.13 Media Type: Panc (Final Volume 500 mL)

7.13.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
L-WRN Conditioned Media	Details in SOP Section 9.0
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236
Recombinant Human FGF-10 (hFGF-10)	PeproTech, Cat#: 100-26; R&D Systems Cat # 345-FG
SB-431542	Selleckchem, Cat#: S1067; R&D Systems, Cat#: 1614
Gastrin	Tocris, Cat#: 3006; R&D Systems, Cat#: 3006

7.13.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		250 mL	
L-WRN Conditioned Media	100%	250 mL	50%
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	1.25 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	5 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	10 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	5 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	500 µL	10 µM
hEGF	50 µg/mL in DPBS	500 µL	50 ng/mL
hFGF-10	25 µg/250 µL 0.1% BSA in DPBS	500 µL	100 ng/mL
SB-431542	10 mM in DMSO	25 µL	500 nM
Gastrin	100 µM (1 mg/4.8 mL) DBPS	50 µL	10 nM

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7.14 Media Type: Prostate (Final Volume 200 mL)

7.14.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
L-WRN Conditioned Media	Details in SOP Section 9.0
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236
Recombinant Human FGF-10 (hFGF-10)	PeproTech, Cat#: 100-26; R&D Systems, Cat#: 345-FG
Recombinant Human FGF-basic (hFGF-2)	PeproTech, Cat#: 100-18B; R&D Systems, Cat#: 233-FB
Prostaglandin E ₂ (PGE ₂)	Tocris, Cat#: 2296; R&D Systems, Cat#: 2296
SB-431542	Selleckchem, Cat#: S1067; R&D Systems, Cat#: 1614
SB-202190	Sigma, Cat#: S7067-5MG; R&D Systems, Cat#: 1264
5 α -Dihydrotestosterone (DHT; 1 mg/mL, 3.4 mM)	Sigma, Cat#: D-073-1ml

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7.14.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Conc.
PDOrg Basic Media		100 mL	
L-WRN Conditioned Media	100%	100 mL	50%
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	0.5 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	2 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	4 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	2 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	200 µL	10 µM
hEGF	50 µg/mL in DPBS	20 µL	5 ng/mL
hFGF-10	25 µg/250 µL 0.1% BSA in DPBS	20 µL	10 ng/mL
hFGF-2	10 µg/mL in 0.1% BSA	20 µL	1 ng/mL
PGE2	20 mM (10 mg/1.42 mL) in DMSO	10 µL	1 µM
SB-431542	10 mM in DMSO	10 µL	500 nM
SB-202190	10 mM (5 mg/1.5 mL DMSO)	200 µL	10 µM
DHT*	3.4 mM Manufacturer-provided Stock solution*; 10 µM Working Stock solution in PDOrg Basic Media	20 µL	1 nM

*DHT stock and working stock preparation recommendations to ensure stability and to maintain reagent in solution. The 3.4 mM manufacturer-provided Stock solution can be maintained for long-term storage. Just before use, make a Working Stock solution by diluting 1:10 twice followed by 1:3.4 to keep volume low and ensure pipetting accuracy (1:340 total) using PDOrg Basic Media for a final Working stock concentration of 10 µM. Use Working Stock solution to prepare Media.

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7.15 Media Type: SCLC (Final Volume 500 mL)

7.15.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
N-acetylcysteine	Sigma, Cat#: A9165-5G
L-WRN Conditioned Media	Details in SOP Section 9.0
Nicotinamide	Sigma, Cat#: N0636-100G
N21-MAX Media Supplement (50X) or B-27 Supplement (50X)	R&D Systems Cat # AR008; Life Technologies, Cat#: 17504044
N-2 MAX Media Supplement (100X) or N-2 Supplement (100X)	R&D Systems Cat # AR009; Life Technologies, Cat#: 17502048
Y-27632 dihydrochloride	Tocris, Cat#: 1254
EGF Recombinant Human Protein (hEGF)	Invitrogen, Cat#: PHG0311; R&D Systems, Cat#: AFL236
Recombinant Human FGF-10 (hFGF-10)	PeproTech, Cat#: 100-26; R&D Systems Cat # 345-FG
Recombinant Human FGF-basic (hFGF-2)	PeproTech, Cat#: 100-18B; R&D Systems, Cat#: 233-FB
Prostaglandin E ₂ (PGE ₂)	Tocris, Cat#: 2296; R&D Systems, Cat#: 2296
SB-431542	Selleckchem, Cat#: S1067; R&D Systems, Cat#: 1614

7.15.2 Prepare Media fresh each week. Several reagents have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock Concentration	Volume	Working Concentration
PDOrg Basic Media		250 mL	
L-WRN Conditioned Media	100%	250 mL	50%
N-acetylcysteine	500 mM in sterile water (81.6 mg/mL)	1.25 mL	1.25 mM
Nicotinamide	1M (1.22 g/10 mL) DPBS	5 mL	10 mM
N21-MAX Media Supplement or B-27 Supplement	50X	10 mL	1X
N-2 MAX Media Supplement or N-2 Supplement	100X	5 mL	1X
Y-27632 dihydrochloride	10 mM in Sterile Water (high grade) (3.2 mg/mL)	500 µL	10 µM
hEGF	50 µg/mL in DPBS	50 µL	5 ng/mL
hFGF-10	25 µg/250 µL 0.1% BSA in DPBS	50 µL	10 ng/mL
hFGF-2	10 µg/mL in 0.1% BSA	50 µL	1 ng/mL
PGE ₂	20 mM (10 mg/1.42 mL) in DMSO	25 µL	1 µM
SB-431542	10 mM in DMSO	25 µL	500 nM

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8.0 CONTRIBUTOR/OTHER MEDIA RECIPES

8.1 Media Type: Cholangiocarcinoma (Final Volume 500 mL)

Contributor: Dr. Nabeel Bardeesy, Massachusetts General Research Institute, MA

8.1.1 Reagents; follow manufacturer's recommendations

Item (Concentration)	Catalog
RPMI-1640, no L-glutamine	ThermoFisher, Cat#: 21870076
HyClone Characterized Fetal Bovine Serum, Heat-inactivated	Cytiva, Cat#: SH30071.03HI
HEPES (1M)	Invitrogen, Cat#: 15630080
L-Glutamine, 200 mM	Invitrogen, Cat#: 25030-081
Pen/Strep, 10000 U/mL	Invitrogen, Cat#: 1514022

8.1.2 Prepare Media fresh each week. Several reagents may have short half-lives, instructions for media preparation should be followed to ensure the best outcome.

Item	Stock solution	Volume	Working Concentration
RPMI-1640, no L-glutamine	--	427.5 mL	
Fetal Bovine Serum	100%	50 mL	10%
HEPES	1M	12.5 mL	25mM
L-Glutamine	200 mM	5 mL	2 mM
Pen/Strep	10000 U/mL	5 mL	100 U/mL

9.0 PREPARATION OF L-WRN CONDITIONED MEDIA

Item	Catalog
L-WRN cells	ATCC, Cat#: CRL-3276

- Manufacturer's Protocol and recommended reagents: <https://www.atcc.org/products/crl-3276>

9.1.1 Prepare L-WRN Conditioned Media (final concentration: 100%) fresh each week.

9.1.2 The PDMR follows the manufacturer's directions for preparation and aliquot of the conditioned media with the following exceptions: (1) do not dilute the final product to 50% final concentration and (2) filter the final product using sterile 0.22 μ m units following the last media collection and centrifugation.

10.0 RECOMMENDED QUALITY CONTROL

10.1 Maintain a record of reagents used to prepare media.

10.2 Document vendors and lot numbers of all media components.

10.3 At lot change-over, parallel new reagents with existing lots prior to placing a new lot into service.

11.0 REFERENCES

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- Sato, T., et al., *Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium*. *Gastroenterology*, 2011. **141**(5): p. 1762-72. <https://www.ncbi.nlm.nih.gov/pubmed/21889923>
- Tuveson Laboratory Protocols, Cold Spring Harbor Laboratory. *Murine and Human Organoid Protocols* (Version: 4/27/2016). Link to protocol: <http://tuvesonlab.labsites.cshl.edu/wp-content/uploads/sites/49/2017/01/20160427-TuvesonOrganoidProtocols.pdf>
- Walsh, A.J., et al., *Quantitative optical imaging of primary tumor organoid metabolism predicts drug response in breast cancer*. *Cancer Res*, 2014. **74**(18): p. 5184-94. <https://www.ncbi.nlm.nih.gov/pubmed/25100563>