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.

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

Table of Contents

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 material 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, 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 REAGENTS & 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 Equipment

4.2.1 50-mL, 25-mL, 10-mL, 5-mL sterile pipettes

4.2.2 Pipetman and sterile tips

4.2.3 0.22 µm, Sterile Filter Unit, 500 mL

4.2.4 Waste container containing 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

5.0 COMPLETE DMEM/F12 MEDIA

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
Advanced DMEM/F12 1X	--	473 mL
Fetal Bovine Serum	--	25 mL
Hydrocortisone	1 mg/mL in 10% EtOH/ultrapure water	200 µL
EGF Recombinant Human Protein	1 mg/mL prepared according to manufacturer's instructions	5 µL
Adenine	2.4 mg/mL in 1mM HCl	5 mL
Pen/Strep or Primocin	10000 U/mL 50 mg/mL	5 mL 1 mL
L-Glutamine	200 mM	5 mL
Y-27632 dihydrochloride*	10 mM in Sterile Water (high grade) (3.84 mg/mL)	500 µL

*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.

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

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 8.0
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) (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) (3.84 mg/mL)	500 µL	10 µM

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) (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) (3.84 mg/mL)	500 µL	10 µM

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 8.0
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) (3.84 mg/mL)	500 µL	10 µM
hEGF	50 µg/mL in DPBS	500 µL	50 ng/mL

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#: PHG0311-100ug; 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) (3.84 mg/mL)	500 µL	10 µM
hEGF	50 µg/mL in DPBS	500 µL	50 ng/mL

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 8.0
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 (PGE2)	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) (3.84 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

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) (3.84 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

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 8.0
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 (PGE2)	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) (3.84 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
SB-431542	10 mM in DMSO	25 µL	500 nM
SB-202190	10 mM (5 mg/1.5 mL DMSO)	500 µL	10 µM

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
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
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) (3.84 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

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 8.0
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 (PGE2)	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) (3.84 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
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

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 8.0
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 (PGE2)	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

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) (3.84 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

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 8.0
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

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) (3.84 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.

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 8.0
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) (3.84 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

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 8.0
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) (3.84 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

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 8.0
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 (PGE2)	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
5α-Dihydrotestosterone (DHT; 1 mg/mL/3.4 mM)	Sigma, Cat#: D-073-1ml

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) (3.84 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.

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 8.0
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 (PGE2)	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) (3.84 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
PGE2	20 mM (10 mg/1.42 mL) in DMSO	25 µL	1 µM
SB-431542	10 mM in DMSO	25 µL	500 nM

8.0 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/en/Products/Cells_and_Microorganisms/By_Tissue/Adipose_Tissue/CRL-3276.aspx#culturemethod	

8.1.1 Prepare L-WRN Conditioned Media fresh each week.

8.1.2 The PDMR follows the manufacturer's directions for preparation and aliquot of the conditioned media. The exception is that the PDMR filters the final product using sterile 0.22 µm units following the last media collection and centrifugation.

9.0 RECOMMENDED QUALITY CONTROL

9.1 Maintain a record of reagents used to prepare media.

9.2 Document vendors and lot numbers of all media components.

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

10.0 REFERENCES

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