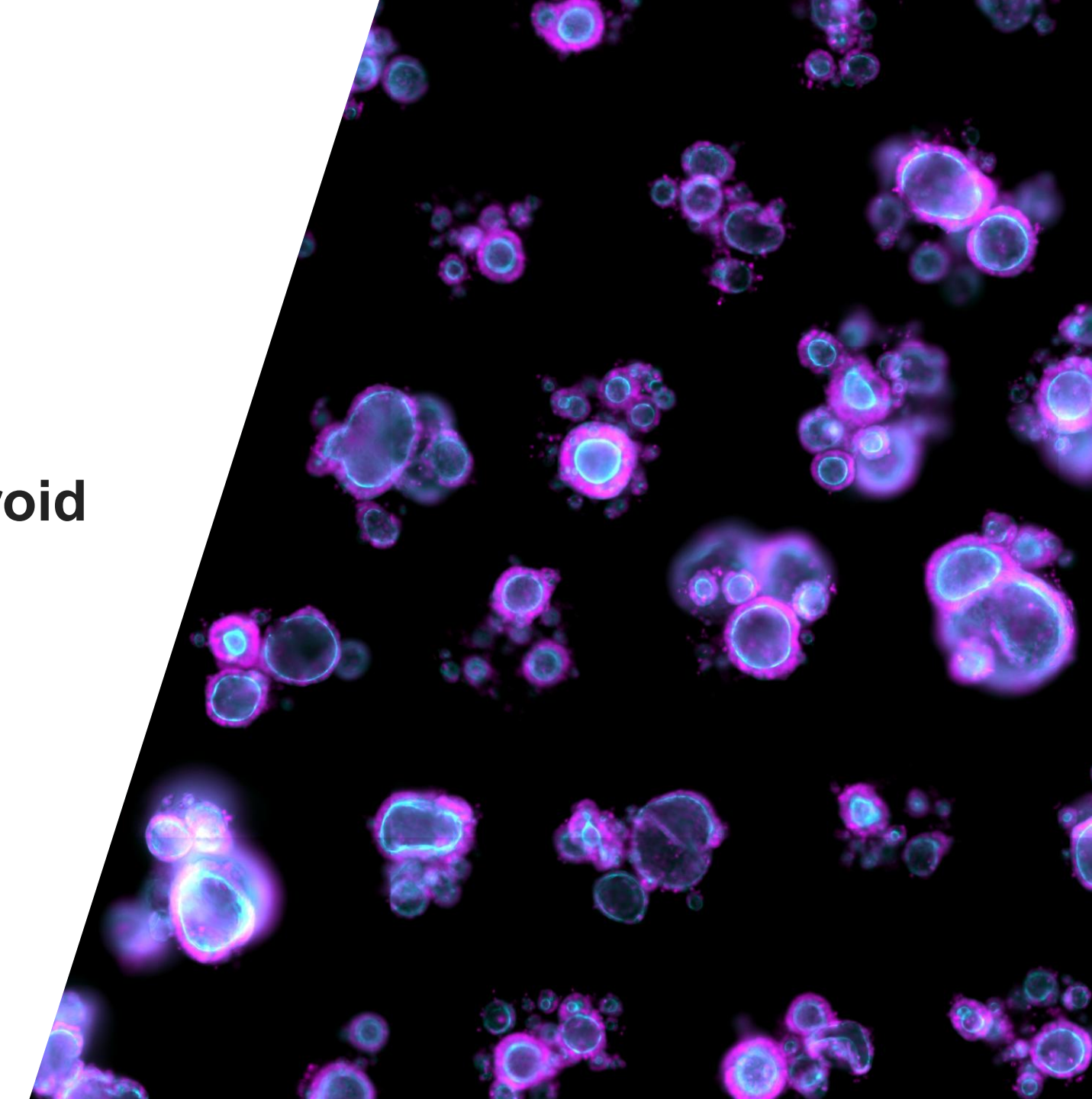
Tissue Dissociation for Tumoroid Derivation

Purpose: Isolate viable cells from patient cancer tissue
Goal: Provide high-quality cells for tumoroid generation

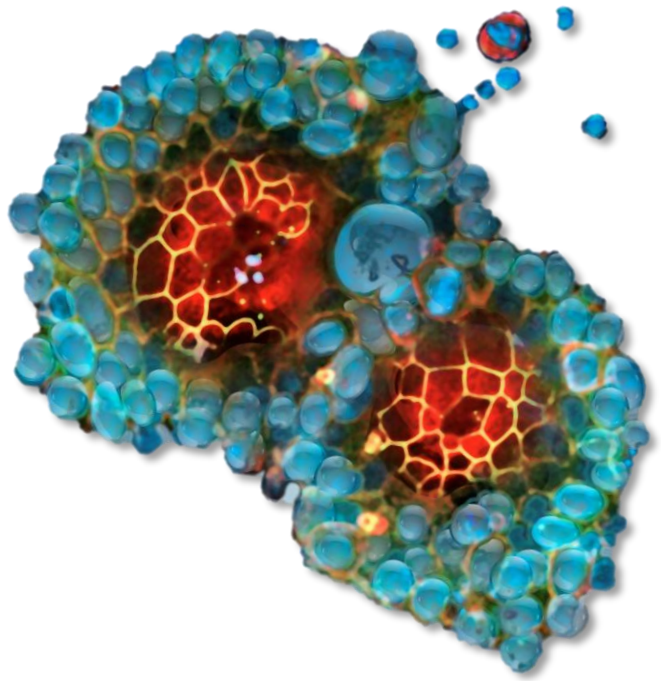


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Introduction

Tumoroids, also known as tumoroid lines, cancer organoids, or patient-derived tumor organoids (PDTOs), are patient-derived cancer cells grown as 3D, self-organized multicellular structures.



Scientific literature suggests tumoroids are a promising tool for drug development studies and precision medicine applications because, compared to traditional 2D immortalized cancer cell lines, they:

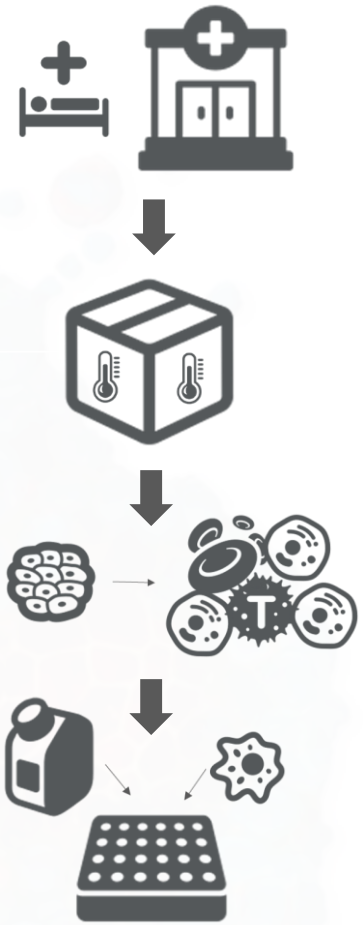
- Better represent patient characteristics
- Support tumor heterogeneity
- Offer more clinically predictive results

This protocol provides guidance on deriving tumoroid lines from fresh tumor tissue samples. Derivation success rates are highly dependent on a number of factors, including cancer indication, cancer cell proportion and viability in the initial sample, and time between sample collection and processing. Therefore, not all samples will be amenable to tumoroid line generation. Additional guidance is available in our derivation application note.

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Overview of process

The following protocol describes transport and processing of fresh tissue for tumoroid generation.



Collect sample

Transport to processing site

Dissociate

Plate cells for tumoroid formation

Upon sample receipt:

1. **Wash and weigh tissue**
2. **Mince into small pieces (mechanical dissociation)**
3. **Perform enzymatic tissue dissociation**
4. **Wash, count, and resuspend cells**
5. **Plate or cryopreserve cells**

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Required materials

Item	Cat. No.
Reagents and buffers	
DPBS, no calcium, no magnesium	14190144
DMEM/F-12, GlutaMAX Supplement	10565018
HBSS, no calcium, no magnesium	14175095
Advanced DMEM/F-12	12634010
TRIzol™ Reagent	15596026
GlutaMAX™ Supplement	35050061
OncoPro™ Tumoroid Culture Medium Kit	A5701201
Y-27632	–
HEPES (1M)	15630130
Primocin®	–
Penicillin-Streptomycin	15140122
B-27™ Supplement (50X), serum free	17504044
Geltrex™ Reduced-Growth Factor Basement-Membrane Matrix, LDEV-free, stem-cell qualified	A1413301
Hibernate™-A Medium	A1247501
Recovery™ Cell Culture Freezing Medium	12648010
PureLink™ Genomic DNA Mini Kit	K182001
TRIzol™ Plus RNA Purification Kit	12183555

Item	Cat. No.
Reagents and buffers	
Collagenase, Type I, powder	17018029
Collagenase, Type III, powder	–
Collagenase, Type IV, powder	17104019
Dispase II, powser	17105041
Hyaluronidase	–
Turbo DNase™ (2 U/µl)	AM2238

Items with a Cat. No. can be purchased through **thermofisher.com**.

Items without a Cat. No. can be purchased from **fishersci.com** or other laboratory suppliers.

Warning: Read the safety data sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. SDSs are available from **thermofisher.com/support**.

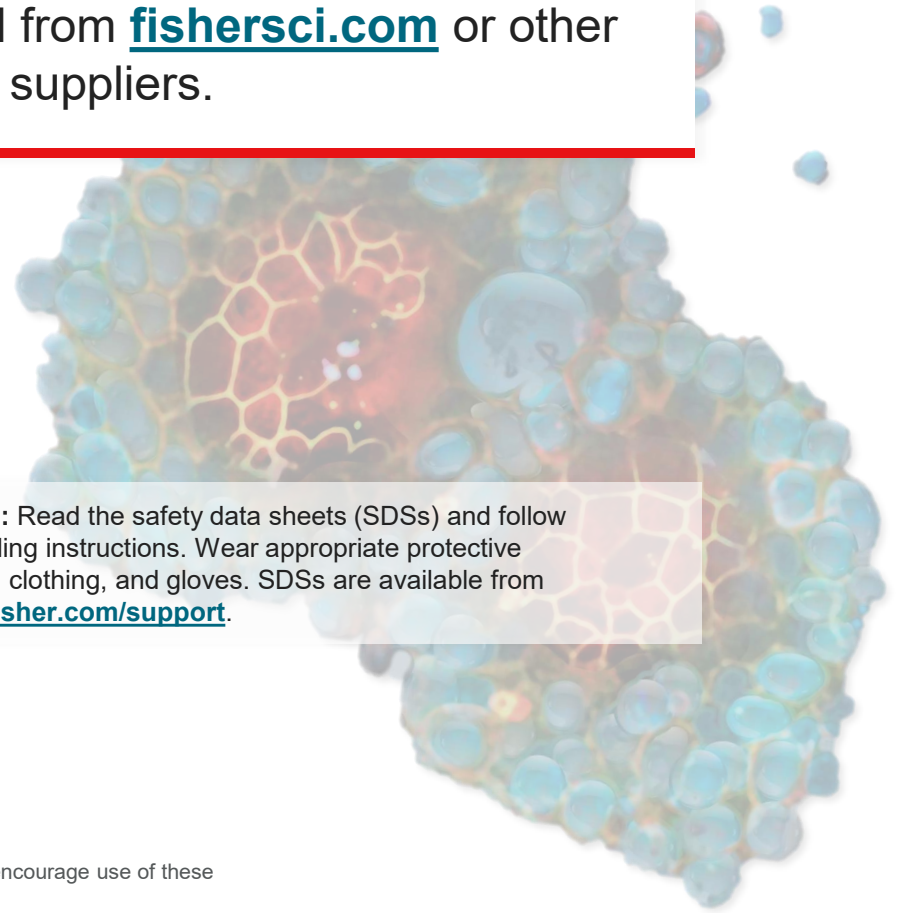
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Required materials

Item	Cat. No.
Consumables	
Snap Cap Low Retention Microcentrifuge Tubes, 1.5 mL	3451PK
Nunc™ Serological Pipettes, 5 mL	170356N
Nunc™ Serological Pipettes, 10 mL	170356N
Nunc™ Serological Pipettes, 50 mL	170356N
Nunc™ 15 mL Conical Sterile Polypropylene Centrifuge Tubes	339650
Nunc™ 50 mL Conical Sterile Polypropylene Centrifuge Tubes	339652
SoftFit-L™ Filtered Pipette Tips in Hinged Racks	2779-HR
Nunc™ Petri Dishes	263991
100 µm mesh cell strainer	–
Nunc™ Non-Treated Multidishes (6 Well)	150239
Equipment	
Orbital shaker	88881103
Centrifuge	–
Microscope	–
Biosafety cabinet	–
Forceps	–
Sterile pestle	–
Cell counter	–
Lab scale	–

Items with a Cat. No. can be purchased through **thermofisher.com**.

Items without a Cat. No. can be purchased from **fishersci.com** or other laboratory suppliers.



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Tissue Transport Buffer: Overview and preparation

Tissue Transport Buffer is designed to retain cell viability during transport. The basal component, Hibernate™-A Medium, is buffered to allow transport and storage at ambient CO_2 levels. Additional components help provide nutrients and antioxidants to support cell function, as well as antimicrobials to protect against contamination from bacteria, fungi, and mycoplasma. The ROCK pathway inhibitor Y-27632 can optionally be added to protect cells from anoikis-mediated cell death.

Note: Transport buffer is aliquoted, sent to site of tissue collection, and stored at 4°C. Upon collection, sample is submerged in Tissue Transport Buffer and shipped to lab for processing on gel packs. Overnight shipment (or shorter) is recommended. Fill transport tubes to leave minimal headspace.

Tissue Transport Buffer

Component	Amount in ml
Hibernate™-A Medium (1x)	239.5 ml
GlutaMAX™ Supplement (100X)	2.5 ml
B-27™ Supplement (50X)	5 ml
Pen-Strep	2.5 ml
Primocin®	0.5 ml
Y-27632 (optional; assumes 10 mM stock solution for final 10 µM concentration)	0.25 ml

Note: Makes 250 ml of Tissue Transport Buffer

Note: Scale volumes to formulate different amounts of Tissue Transport Buffer

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Tissue Processing Buffer: Overview and preparation

Tissue Processing Buffer creates a supportive environment during dissociation of tissue samples by helping to provide essential nutrients, stabilizing the pH during processing, and serving as a source of calcium required for collagenase activity. It also includes a mix of antimicrobial agents to protect against contamination. The ROCK pathway inhibitor Y-27632 is added to protect cells from anoikis-mediated cell death as single cells are released.

Note: Formulate complete Tissue Processing Buffer on the day of tissue receipt. 50-100 ml of Tissue Processing Buffer are typically required for each sample received.

Tissue Processing Buffer

Component	To formulate 100 ml	To formulate 50 ml
Advanced DMEM/F-12 Medium	93.7 ml	47.9 ml
GlutaMAX™ Supplement	1 ml	0.5 ml
HEPES (1M stock)	1 ml	0.5 ml
B-27™ Supplement (50x)	2 ml	1 ml
Pen-Strep	2 ml	1 ml
Primocin® (50mg/ml)	0.2 ml	0.1 ml
Y-27632 (assumes 10 mM stock solution for final 10 µM concentration)	0.1 ml	0.05 ml

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Enzyme Mix: Overview and preparation

The Enzyme Mix should gently dissociate tumor tissue and release viable cells (mixture of single cells and small cell clusters) that can form tumoroids in culture. Over-dissociation of tissue could result in poor performance in culture, even if cell viability is high immediately following tissue processing.

Note: Formulate Enzyme Mix immediately prior to tissue dissociation.

Enzyme Mix

Component	Amount in ml
Tissue Processing Buffer	2.5 ml
Collagenase Type IV	To 20-200 U/ml activity
DNase I	50 U/ml
Other enzymes as needed	Empirically determine

Note

Enzyme mixtures may need to be optimized for different cancer indications. We recommend starting with 20-200 U/ml of collagenase type IV and 50 U/ml DNase I. Enzyme concentration can be modified if tissue is over- or under-dissociated. Additional dissociation enzymes could also be added to the Enzyme Mix. Commonly used dissociation enzymes include collagenase type I-IV (0-200 U/ml), hyaluronidase (0-200 U/ml), elastase (0-20 U/ml), dispase (0-10 U/ml), and DNase I (0-100 U/ml). **Please see following slide for recommended enzyme mixtures for select cancer indications.**

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Enzyme Mix: Indication-specific recommendations

The Enzyme Mix should gently dissociate tumor tissue and release viable cells (mixture of single cells and small cell clusters) that can form tumoroids in culture. Over-dissociation of tissue could result in poor performance in culture, even if cell viability is high immediately following tissue processing.

Note: Formulate Enzyme Mix immediately prior to tissue dissociation.

Colorectal cancer

Component	Amount in ml
Tissue Processing Buffer	2.5 ml
Collagenase Type I	20 U/ml
Collagenase Type III	50 U/ml
Dispase	1 U/ml
DNase I	50 U/ml

Lung cancer

Component	Amount in ml
Tissue Processing Buffer	2.5 ml
Collagenase Type I	80 U/ml
Hyaluronidase	50 U/ml
Dispase	1 U/ml
DNase I	100 U/ml

Breast cancer

Component	Amount in ml
Tissue Processing Buffer	2.5 ml
Collagenase Type III	150 U/ml
Dispase	1 U/ml
DNase I	50 U/ml

Endometrial cancer

Component	Amount in ml
Tissue Processing Buffer	2.5 ml
Collagenase Type III	5 U/ml
Collagenase Type IV	5 U/ml
Dispase	7 U/ml
DNase I	50 U/ml

Note

Enzyme mixtures may need to be optimized for different cancer indications. To generate the recommendations above, collagenase type I-IV (0-200 U/ml), hyaluronidase (0-200 U/ml), elastase (0-20 U/ml), dispase (0-10 U/ml), and DNase I (0-500 U/ml) were empirically tested across donor samples

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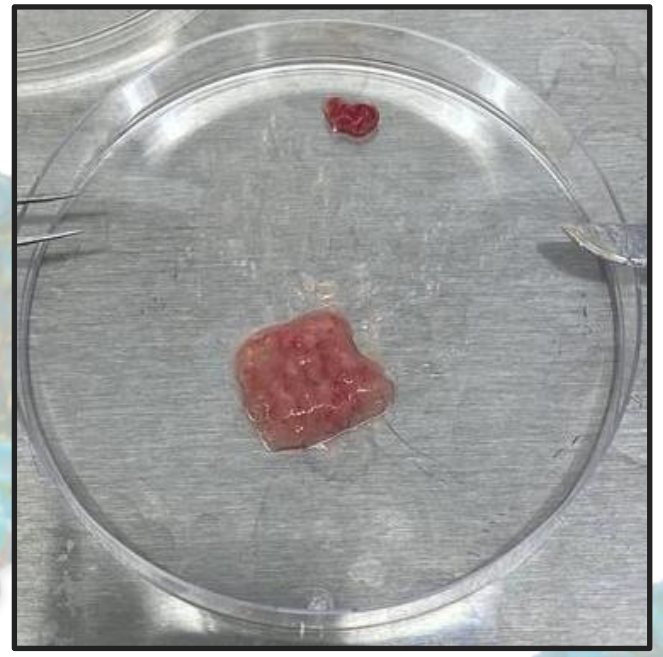
Weigh and wash tissue

1. Weigh an empty 50 ml tube. Record dry weight of tube.
   Note: Weighing tube prior to tissue processing enables calculation of cell yield per mg tissue.
2. In a biosafety hood, pour tissue and transport medium from transport container into a sterile petri dish.
3. Rinse the original transport container with more 5 ml Tissue Processing Buffer and add rinse to the petri dish containing tissue pieces.
4. Transfer tissue and all media from petri dish to a **new** 50 ml Tube using sterile forceps and serological pipettes.
   Note: This is not the tube that was weighed in Step 1.
5. Use 5 ml fresh Tissue Processing Buffer to rinse out the petri dish; add rinse to tube containing the tissue.
6. Add fresh Tissue Processing Buffer to 50 ml tube to bring total volume to 40 ml (if volume in tube < 40 ml).
7. Centrifuge at 100x*g* for 5 minutes at 4°C. Aspirate supernatant.
8. Add 20 ml Tissue Processing Buffer and invert tube to wash tissue.
9. Centrifuge at 100x*g* for 5 minutes at 4°C. Aspirate supernatant, leaving ~3-5 ml behind.
10. Pour tissue and remaining buffer into new, sterile petri dish.

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Mince and dissociate tissue

1. Mince tissue into 2-4 mm fragments using sterile scalpel(s) and forceps.
   Note: If samples are required for subsequent nucleic acid isolation, set fragments aside (see next slide).
2. Use forceps to transfer minced tissue into the **previously weighed** 50 ml conical tube. Weigh tube again (tissue weight = new weight – dry weight).
3. Add 2.5 ml Enzyme Mix to 50 ml conical tube containing tissue.
   Note: Use 5 ml Enzyme Mix for large tissues.
4. Seal tube with parafilm around cap.
5. Incubate at 37°C, 5% CO_2 for 1 hour. Shake or swirl often (or use incubator-compatible orbital shaker set at 120 rpm to provide agitation during dissociation).



Note

The larger tissue piece in the image above is representative of the size of a large surgical resection sample. The smaller tissue piece above is an approximately 2-4 mm fragment, post-mincing, used for dissociation and nucleic acid isolation.

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Optional: Collect tissue fragments for subsequent nucleic acid isolation

For RNA isolation

1. Use forceps to transfer tissue fragment(s) to 1.5 ml low retention microcentrifuge tube.
2. Add 500 µl TRIzol™ Reagent and macerate with a sterile pestle.
3. Add additional 500 µl TRIzol™ Reagent and triturate thoroughly using a P1000 pipet tip.
4. Store at -20°C until RNA is isolated (using, for example, TRIzol™ Plus RNA Purification Kit).

Note: If excess Tissue Processing Buffer carries over during transfer of tissue fragments, centrifuge samples at 100x*g* for 5 min at 4°C and aspirate supernatant prior to adding TRIzol™ Reagent.

For DNA isolation

1. Use forceps to transfer tissue fragment(s) to 1.5 ml low retention microcentrifuge tube.
2. Close tube and flash-freeze in liquid nitrogen (if available).
3. Store at -80°C until DNA is isolated (using, for example, PureLink™ Genomic DNA Mini Kit).

Note: If excess Tissue Processing Buffer carries over during transfer of tissue fragments, centrifuge samples at 100x*g* for 5 min at 4°C and aspirate supernatant prior to flash freezing tissue.

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Wash cells following tissue dissociation

1. Add 10 ml HBSS(-/-) supplemented with 10 µM Y-27632 to the 50 ml tube containing tissue and dissociation enzymes.
2. Mix well and transfer contents to a new 15 ml conical tube.
3. Centrifuge tube at 200x*g* for 5 minutes at 4°C.
   Note: If cells do not pellet, invert tube several times and then repeat centrifugation step.
4. Transfer supernatant to new 15 ml conical tube and centrifuge at 200x*g* for 5 minutes at 4°C. Aspirate supernatant.
5. Resuspend and combine pellets in 10 ml total HBSS(-/-) supplemented with 10 µM Y-27632.
6. Centrifuge tube at 200x*g* for 5 minutes at 4°C. Aspirate supernatant.
7. Resuspend cells in 10 ml of OncoPro™ Tumoroid Culture Medium supplement with 10 µM Y-27632.
8. Pass cell solution through a 100 µm cell strainer. Transfer contents to a new 15 ml conical tube
9. Centrifuge 200xg for 5 minutes at 4°C, then aspirate supernatant.
10. Resuspend pellet in 5 ml of OncoPro™ Tumoroid Culture Medium supplement with 10 µM Y-27632.

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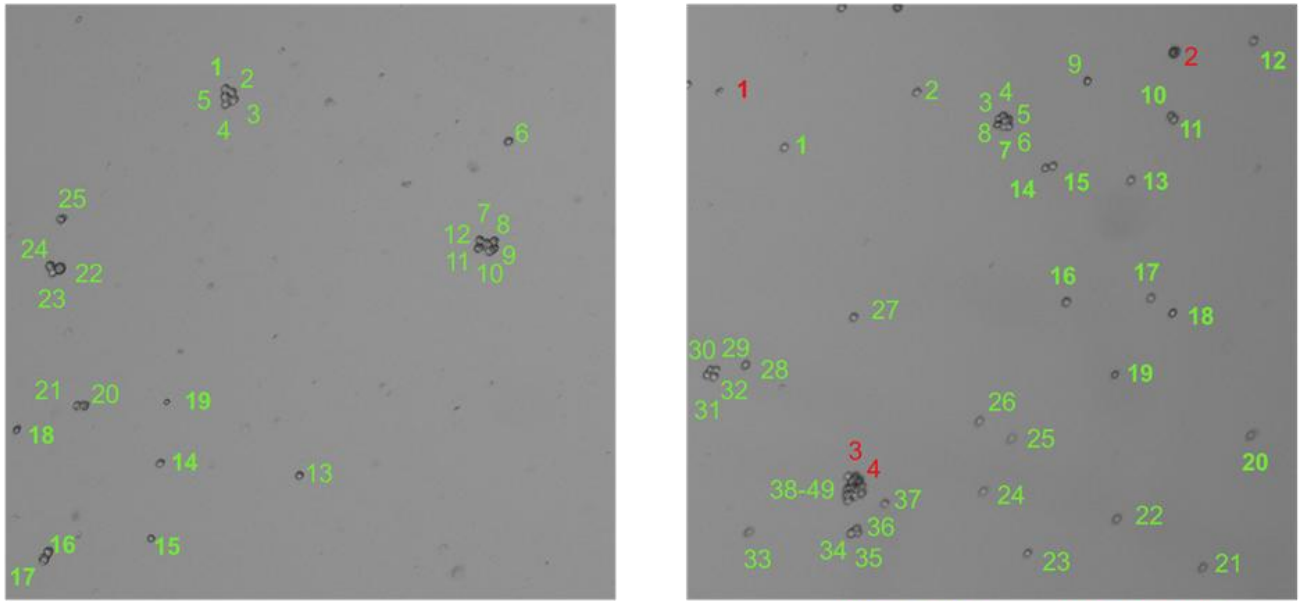
Count cells following tissue dissociation

1. Add 400 µl OncoPro Tumoroid Culture Medium supplemented with 10 µM Y27632 to a new 1.5 ml microcentrifuge tube.

2. Swirl tube containing cell suspension, then use a pre-wetted tip to triturate 5 times. Draw up 400 µl of cell suspension and place into the 1.5 ml microcentrifuge tube prepared in Step 1.

3. Count cells diluted 1:2 using a hemocytometer or automated cell counter.

Representative images of dissociated cells. Both single cells and small cell clusters will be present following dissociation.



Count cells in clusters and use decluster algorithms as much as possible to detect individual cells. **Live cells** **Dead cells**

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Resuspend cells and initiate tumoroid culture

1. Calculate the average viable cells/ml from replicate cell counts.
2. Determine the total number of cells remaining after counting:

   Total viable cells = 4.6 ml * (average viable cells/ml)
3. Centrifuge tube containing cells at 400x*g* for 5 min at 4°C. Carefully aspirate supernatant
4. Resuspend cells to a density of 250,000 viable cells/ml in OncoPro Tumoroid Culture Medium supplemented with 10 µM Y-27632.
5. Plate cells at 250,000 viable cells/ml in OncoPro Tumoroid Culture Medium supplemented with 10 µM Y-27632 in non-treated polystyrene cell culture plates or dishes.

   Note: Scale format as dictated by cell yields. We frequently plate 500,000 cells/well in non-treated 6-well plates, with 2 ml volume per well.
6. Drip in Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix to achieve a final concentration of 2% v/v in suspension.
7. Place cells in 37°C, 5% CO_2 humidified incubator for culture.

Note

If needed, dissociated cells can be cryopreserved in Recovery™ Cell Culture Freezing Medium or other cryopreservative for later culture. Cell viability, yield, and derivation success rate may be decreased when using cryopreserved cells instead of freshly dissociated cells, and derivation timelines may be extended.

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Optional: Collect dissociated cells for subsequent nucleic acid isolation

For RNA isolation

1. After resuspension, transfer 250-500K viable cells to 1.5 ml low retention microcentrifuge tube.
2. Centrifuge at 400x*g* for 5 min at 4°C and aspirate supernatant.
3. Add 700 µl TRIzol™ Reagent to pellet and triturate thoroughly using a P1000 pipet tip.
4. Store at -20°C until RNA is isolated (using, for example, TRIzol™ Plus RNA Purification Kit).

For DNA isolation

1. After resuspension, transfer 250-500K viable cells to 1.5 ml low retention microcentrifuge tube.
2. Centrifuge at 400x*g* for 5 min at 4°C. Aspirate supernatant and resuspend pellet in 1 ml DPBS (-/-).
3. Centrifuge at 400x*g* for 5 min at 4°C. Aspirate supernatant and resuspend pellet in 1 ml DPBS (-/-) to wash again.
4. Centrifuge at 400x*g* for 5 min at 4°C, then aspirate supernatant, leaving as little liquid as possible.
5. Store cell pellet at -80°C until DNA is isolated (using, for example, PureLink™ Genomic DNA Mini Kit).

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Tumoroid formation and initial culture

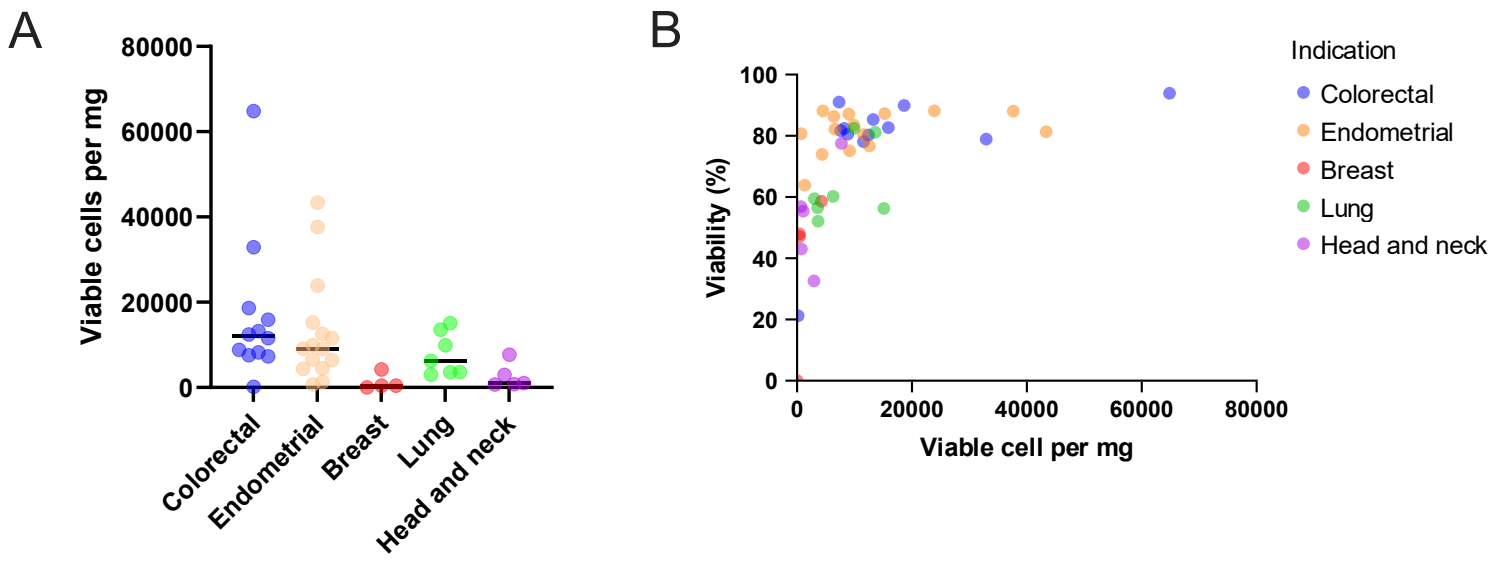
1. Incubate cells in suspension for 2-7 days to promote cell aggregation – target 2-3 days for most donors.
2. After incubation in suspension, collect all tumoroids and media and move to fresh conical tube.
3. Wash culture vessel with cold DMEM/F12, GlutaMAX™ supplement and add wash media to cell suspension.
4. Centrifuge at 400x*g* for 5 min at 4°C, then aspirate supernatant.
5. Follow OncoPro Tumoroid Culture Medium protocol to embed cell clusters in Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix domes.
   Note: Target 4 domes seeded per well of 6-well plate initially plated. Do not dissociate clusters prior to embedding.
6. Follow OncoPro Tumoroid Culture Medium protocol to feed and culture cells using embedded culture method until stable cell expansion is observed – typically requires 3-5 passages, with passaging every 5-14 days.
   Note: Split tumoroids when size reaches ~200-300 µm in diameter. Most tumoroid lines are split every 7 days.
7. After stable growth is observed, split tumoroids and re-seed following suspension protocol for scale up and routine culture.

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Representative results

Cell yields following tissue dissociation vary by indication and donor

Colorectal, endometrial, breast, lung, and head and neck surgical resection samples were received, weighed, and dissociated by mechanical and enzymatic methods. Viable cell yield was normalized to initial tumor mass and showed strong donor dependence. On average, colorectal and endometrial samples yielded more viable cells per unit mass than other indications tested. Our more successful dissociations yielded cell viability of ~80% post-dissociation.



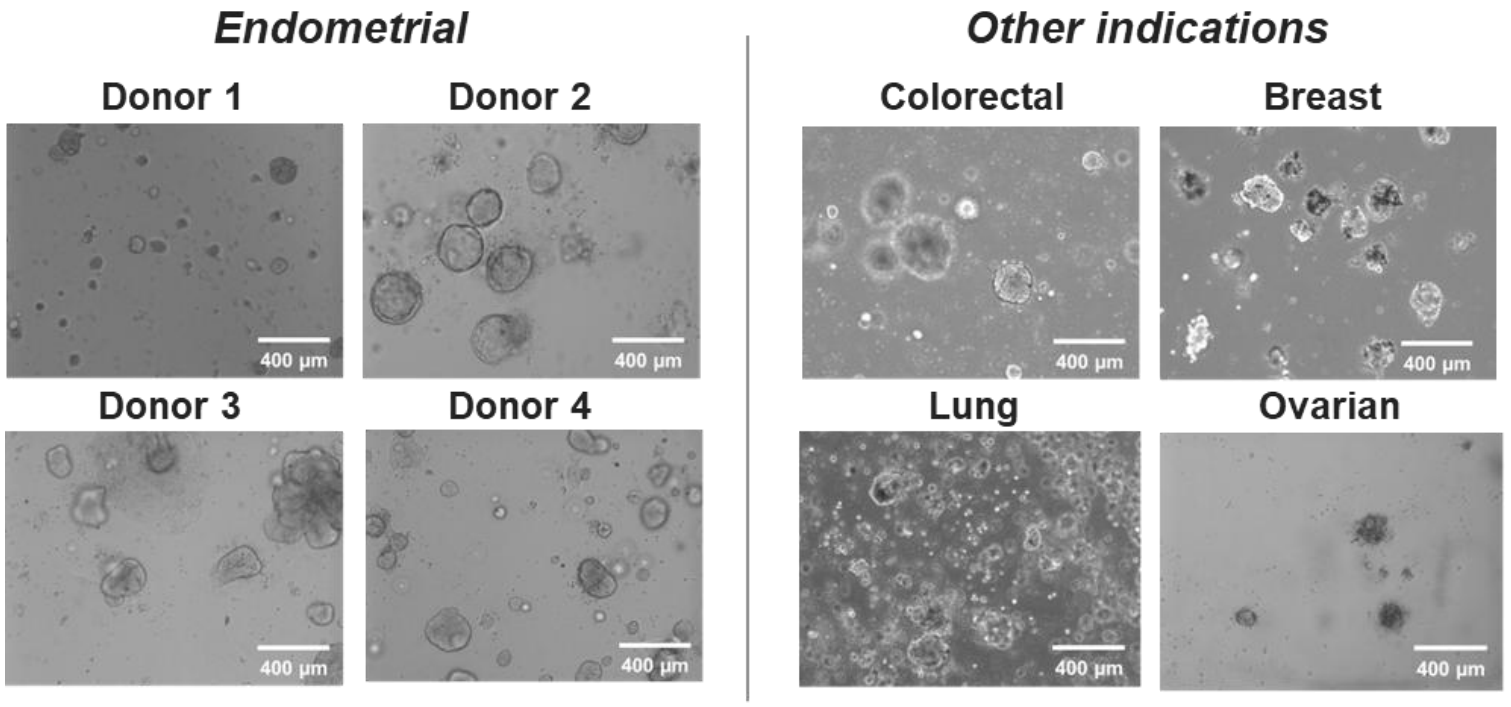
Donor-specific cell yields and viabilities following dissociation of solid tumor resection samples. (A) Viable cells per mg tissue measured immediately following tissue dissociation. Mean viable cells per mg is displayed for each indication. (B) Plot of cell viability post-dissociation (%) vs. viable cell yield after tissue dissociation (normalized by tissue mass). Each point represents one donor in plots.

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Representative results

Formation of tumoroids within 7 days of tissue dissociation in OncoPro™ Tumoroid Culture Medium

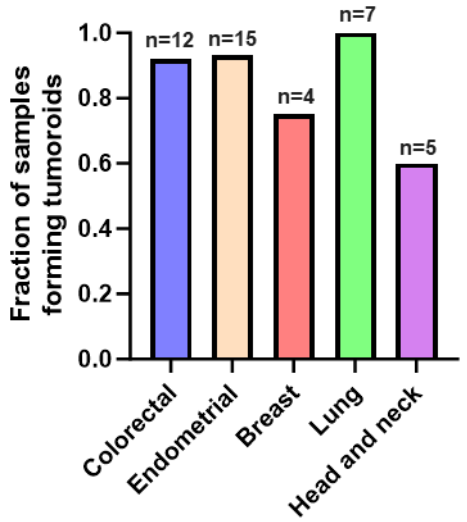
Imaging of tumoroids formed 7 days after seeding freshly dissociated tumor cells demonstrated that ~90% of samples tested formed 3D structures upon culture in OncoPro™ Tumoroid Culture Medium.



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Representative results

Formation of tumoroids within 7 days of tissue dissociation in OncoPro™ Tumoroid Culture Medium



Tumoroid formation after 7 days in culture in OncoPro Tumoroid Culture Medium. Plates were visually inspected and counted as having generated tumoroids if 3D structures were observed. The fraction of samples (one sample per donor) in which tumoroids formed was calculated for each indication.

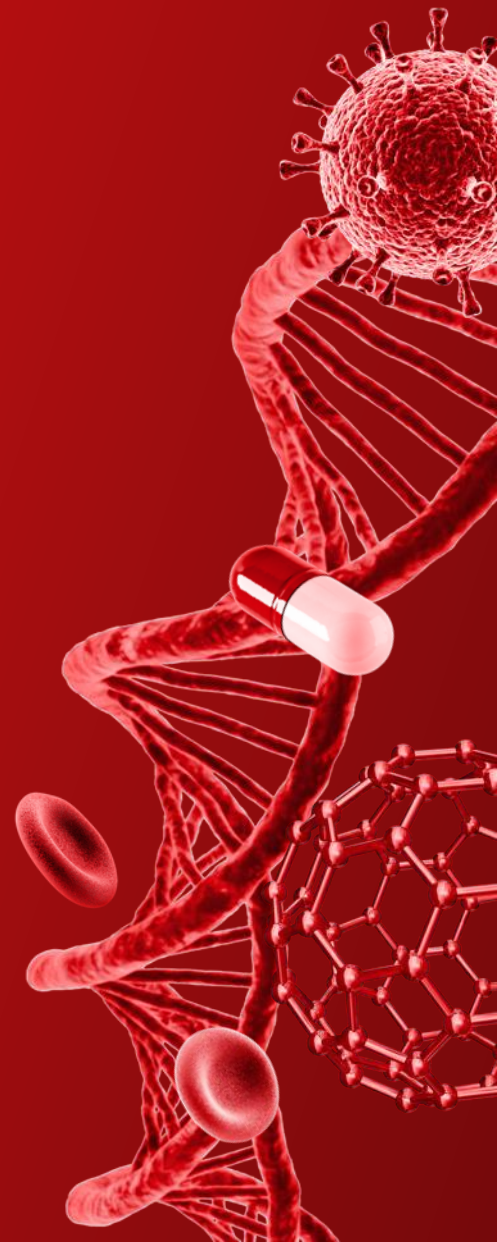
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Troubleshooting

Problem/question	Solution/answer
Tissue does not fully dissociate	Ensure that Enzyme Mix is formulated on the day of dissociation and agitate tissue during incubation at 37°C and 5% CO_2. Consider the use of commercial instruments that provide agitation and heating during enzymatic dissociation. Non-digested tissue fragments can be placed in fresh Enzyme Mix following the 1-hour initial dissociation to attempt further dissociation.
Low cell viability after dissociation	Viability can vary based on tissue sample. For maximum cell yield, process sample as soon as possible following collection. Decrease enzyme concentrations in Enzyme Mix to avoid over-digestion of tissue.
Low cell yield after dissociation	Cell yield can vary widely based on cancer indication and characteristics of individual samples. For maximum cell yield, process sample as soon as possible following collection. Ensure that cells pellet during centrifugation steps and are not lost during supernatant aspiration. Employ declustering algorithms on automated cell counters to count individual cells present in cell clusters.
Clogged cell strainer	Be sure tissue is well minced, and no large chunks are present. Use new strainer, if needed.
Tumoroids not forming properly	Confirm that correct initial seeding density was used. Results can vary based on tissue sample.
Cells do not pellet well after centrifugation	Resuspend visible pellet, if any, and spin down tube again at same settings.
Abundance of red blood cells in dissociated sample	Trim and discard any clear fat and/or blood vessels from sample prior to enzymatic dissociation. Remaining red blood cells will lyse during tumoroid culture and be cleared over the first weeks in culture. Cells may be treated with a red cell lysis buffer prior to plating in OncoPro™ Tumoroid Culture Medium, though this treatment may negatively impact initial tumoroid viability.

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Thank you



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