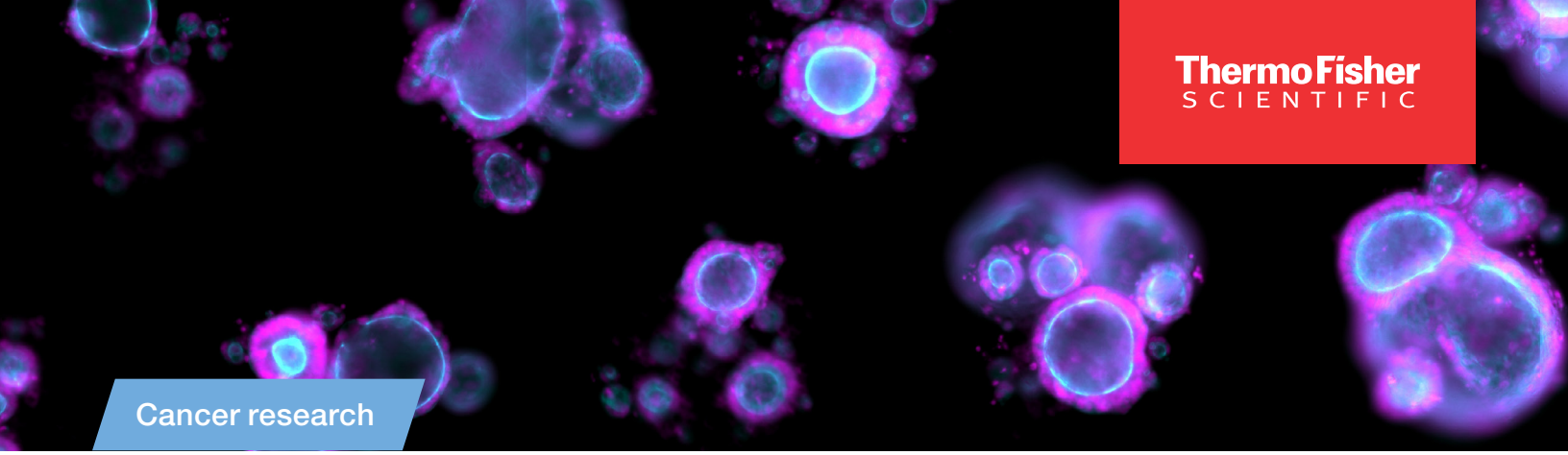

Cancer research

Five steps to establishing tumoroid models for cancer research

This guide provides an overview of the workflow for developing tumoroid models for cancer research, and incorporates tips and tricks from our scientists.

Tumoroids are patient-derived cancer cells grown in 3D that self-organize into multicellular structures. Compared to traditional 2D cancer cell lines, tumoroids better reflect the mutational status, gene expression levels, and phenotypes observed in patient tumors. This makes tumoroid models a vital bridge between laboratory models and clinical research.

The process of developing these models involves sourcing tumoroid lines, expanding and banking the tumoroids, characterizing tumoroid cultures, engineering the models as desired, and leveraging the models for downstream applications of interest. This guide highlights the five key steps to establishing tumoroid models to enable advanced cancer research.

Source

- OncoPro Tumoroid Cell Lines
- Hibernate-A Medium
- Gibco Collagenase Type I–IV
- Gibco Hanks' Balanced Salt Solution
- TrypLE Express Enzyme

Culture

- OncoPro Tumoroid Culture Medium Kit
- Geltrex Basement Membrane Matrix
- Nunc Non-treated Flasks and Multidishes
- Nunc 15 mL and 50 mL Conical Tubes
- StemPro Accutase Cell Dissociation Reagent
- Recovery Cell Culture Freezing Medium
- Gibco DPBS
- SoftFit-L Filtered Pipette Tips in Hinged Racks

Characterize

- EVOS M7000 Imaging System
- Oncomine Comprehensive Assay v3
- Ion AmpliSeq Transcriptome Human Gene Expression Kit
- Attune NxT Flow Cytometer
- BlockAid Blocking Solution
- Invitrogen Alpaca Nano secondary antibody
- Ion Chef Instrument
- Ion GeneStudio S5 Prime System

Engineer

- Neon NxT Electroporation System
- iBlot 3 Western Blot Transfer Device
- iBright CL1500 Imaging System
- Attune NxT Flow Cytometer
- CTS LV-MAX Lentiviral Production System
- *Silencer* GFP siRNA

Assay

- Gibco Human Plasma-Like Medium
- PrestoBlue HS Cell Viability Reagent
- Varioskan LUX Multimode Microplate Reader
- CellEvent Caspase-3/7 detection reagent
- CellInsight CX7 LZR Pro High-Content Screening Platform
- SYTOX Deep Red Nucleic Acid Stain
- Image-iT TMRM Reagent
- CyQUANT LDH Cytotoxicity Assay

Source: Derive or procure tumoroid lines

Tumoroids can be derived from tissue samples or patient-derived xenograft tumors, or established lines can be obtained commercially. To maximize the success of deriving a tumoroid line, tumor tissue from surgical resections is stored in cold transport medium, such as Gibco™ Hibernate™-A Medium, and processed within 24 hours of excision. Upon arrival in the laboratory, the tumor tissue is minced with a scalpel, washed in a buffer, such as Gibco™ Hanks' Balanced Salt Solution supplemented with antibiotics, and dissociated using the appropriate enzymatic dissociation reagent for the particular tissue type. After dissociation, cells are separated from undigested tissue with a cell strainer, washed, and plated in complete Gibco™ OncoPro™ Tumoroid Culture Medium with 2% basement membrane extract (BME) to form small clusters in suspension, and then embedded in BME domes for initial expansion until stable proliferation rates are obtained. Alternatively, tumoroid lines may be established by plating dissociated cells directly into suspension or embedded formats. Once a consistent cell proliferation rate is established, suspension culture is suggested for scale-up of cultures for banking and subsequent routine culture. Generally, tumoroid lines will grow in both suspension and embedded culture, though some lines exhibit faster growth using one method over the other.

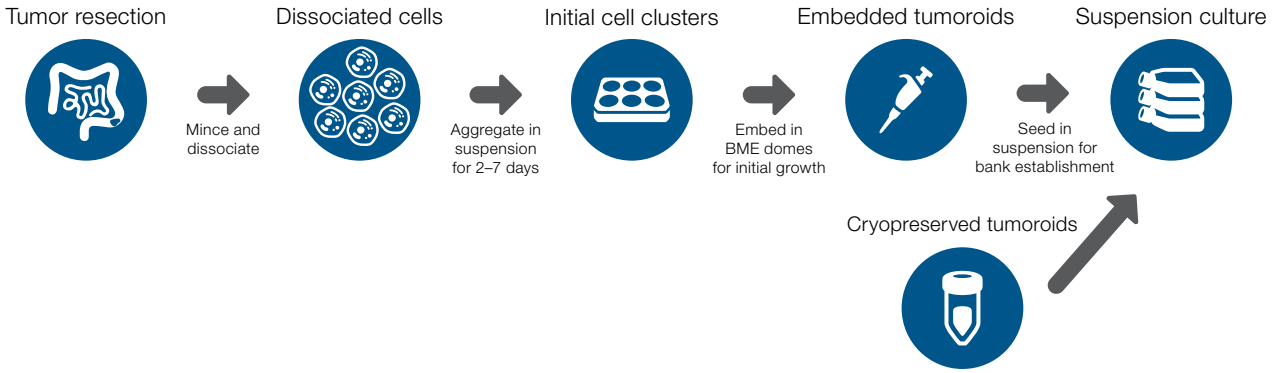

Figure 1. Workflow for initiating tumoroid cultures. Tumoroids can be generated from surgical resections or sourced as established, cryopreserved tumoroid lines.

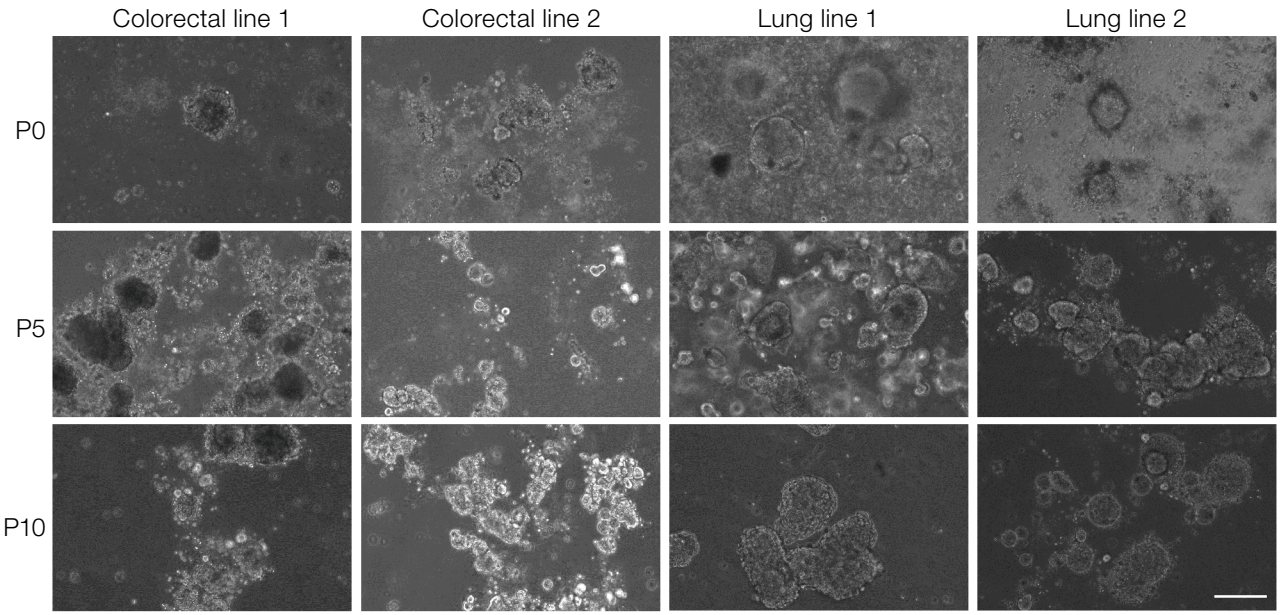

Figure 2. Derivation and maintenance of tumoroid cultures. Representative images of tumoroids cultured in OncoPro Tumoroid Culture Medium at passage (P) 0, 5, and 10. Passage 0 represents only 1–2 weeks of culture. P0 and P5 images represent a mix of embedded and suspension samples. All P10 images are suspension cultures (scale bar = 200 µm).

Tips and tricks

- Antibiotics should be used to help reduce risk of contamination of tumoroid cultures from the surgical resection.
- Embedded culture is recommended until tumoroid cells are proliferating consistently. Tumoroid lines can then be transitioned to suspension culture for routine culture, scale-up, and establishment of a cell bank.
- Tumoroid culture derivation success rates are dependent on several factors, including quality of the tissue, time between resection and culture, and cancer indication and/or subtype. Additionally, tumoroid line derivation usually takes 6–12 weeks but can take longer. To get started with tumoroids faster, obtaining established tumoroid lines from a reliable source is recommended.
- In many cases, the number of viable cells may decline during initial passages when deriving new tumoroid lines from a tissue. If derivation is successful, tumoroids should begin expanding in subsequent passages, and scale-up can be performed.
- Samples should be characterized upon culture initiation and after establishing cryopreserved master banks. Additionally, when multiple tumoroid cultures are established concurrently, samples from master banks should be thawed, cultured for 3–4 passages, and profiled to ensure that no contamination with other tumoroid lines has occurred.

Product highlights

- **Gibco™ Collagenase Type IV (powder)**—The appropriate type and concentration of dissociation reagent for a tissue is dependent on cancer indication. Collagenase Type IV is generally a safe choice because of its relatively gentle action, which can help avoid over-dissociation of the tissue. Over-dissociation can affect cell viability and successful establishment of a tumoroid line.

Culture: Expand tumoroid cultures, and cryopreserve banks

After tumoroid lines are sourced, the next step involves long-term *in vitro* propagation to enable various downstream assays. Depending on cell growth rates, the lines are passaged approximately once every 4–14 days to ensure continuous cell proliferation. During each passage, tumoroids are dissociated to form a mixture of single cells and small cell clusters. With the OncoPro Tumoroid Culture Medium Kit, tumoroids can be propagated in embedded or suspension formats. In the embedded format, tumoroids are embedded in basement membrane extract (BME) and cultivated as domes. In the suspension format, tumoroids are free-floating in complete medium with 2% BME. The choice between these two formats is largely dependent on user preference. However, compared to the embedded format, suspension culture is more user-friendly, cost-effective, scalable, and automation-compatible. For routine culture, it is therefore recommended that tumoroids are cultured in suspension format in a non-treated culture vessel (Figure 3). With either format, it is recommended to change the medium every 2–3 days. If desired, the tumoroids can also be banked for future use.

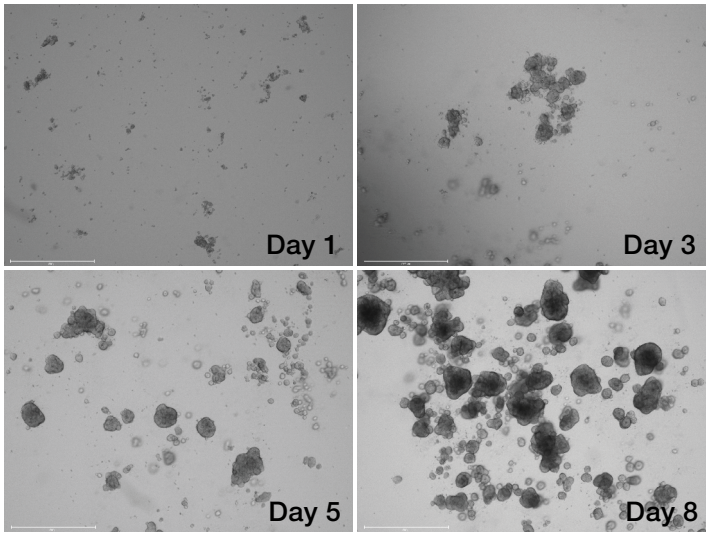

Figure 3. Growth of tumoroids in suspension culture over several days. Images were captured using an Invitrogen™ EVOS™ M7000 Imaging System (scale bar = 1,000 µm).

Tips and tricks

- Use cold medium and wash buffers during passaging to help wash BME from tumoroid cultures prior to dissociation of the tumoroids.
- For maximum recovery of cells during the passaging process, pre-wet serological pipettes and micropipette tips with complete medium before handling tumoroids. This helps ensure that the tumoroids do not stick to the inside of the pipette or tip.
- Dissociate tumoroids to a mixture of single cells and small cell clusters during passaging to help maintain cell viability; avoid over-dissociation.
- Bank at ≥2 million cells per vial in Gibco™ Recovery™ Cell Culture Freezing Medium to maximize post-thaw cell viability.
- Always handle one line at a time and spray down the shaft of your micropipettes with 70% ethanol or isopropanol to reduce the risk of tumoroid line cross-contamination.

Product highlights

Gibco™ OncoPro™ Tumoroid Culture Medium Kit—Medium and supplements required for culturing tumoroids are packaged together in an easy-to-use kit. This medium is compatible with both scale-up and automation-friendly suspension culture, as well as embedded culture, depending on user preference and needs.

Gibco™ StemPro™ Accutase™ Cell Dissociation Reagent—Tumoroids are sensitive to harsh dissociation methods. Use of the StemPro Accutase reagent helps ensure gentle but effective dissociation of tumoroids into small cell clusters, while maintaining high cell viability.

Services

Our research services team will generate your 3D model using the OncoPro Tumoroid Culture Medium Kit. Customers will receive milestone reports with results that include optimized culture conditions such as seeding density, extracellular matrix conditions, and more.

Characterize: Assess growth, gene expression, and protein expression

Characterization of tumoroids is used to compare established lines to initial tumor samples, to measure relevant characteristics (e.g., mutations, gene expression, and protein expression levels), and to monitor stability during culture and expansion. After lines are sourced, mutational and gene expression profiles can be compared to the original tissue or an earlier passage (Figure 4). A high correlation between these profiles demonstrates that the lines are maintaining their patient-specific characteristics. During routine culture, tumoroid morphology, tumoroid size, cell viability, and doubling time can be assessed between passages (Figure 5). Frequent qualitative evaluation of tumoroid morphology using microscopy is recommended; a change in morphology can be an early indicator of culture drift or cross-contamination with another tumoroid line. The tumoroid size also helps determine the timeline for cell passaging. Finally, protein marker expression can be used to compare established tumoroids to original tumor material (Figure 6) or to assess the stability of protein expression levels between passages or upon experimental perturbations.

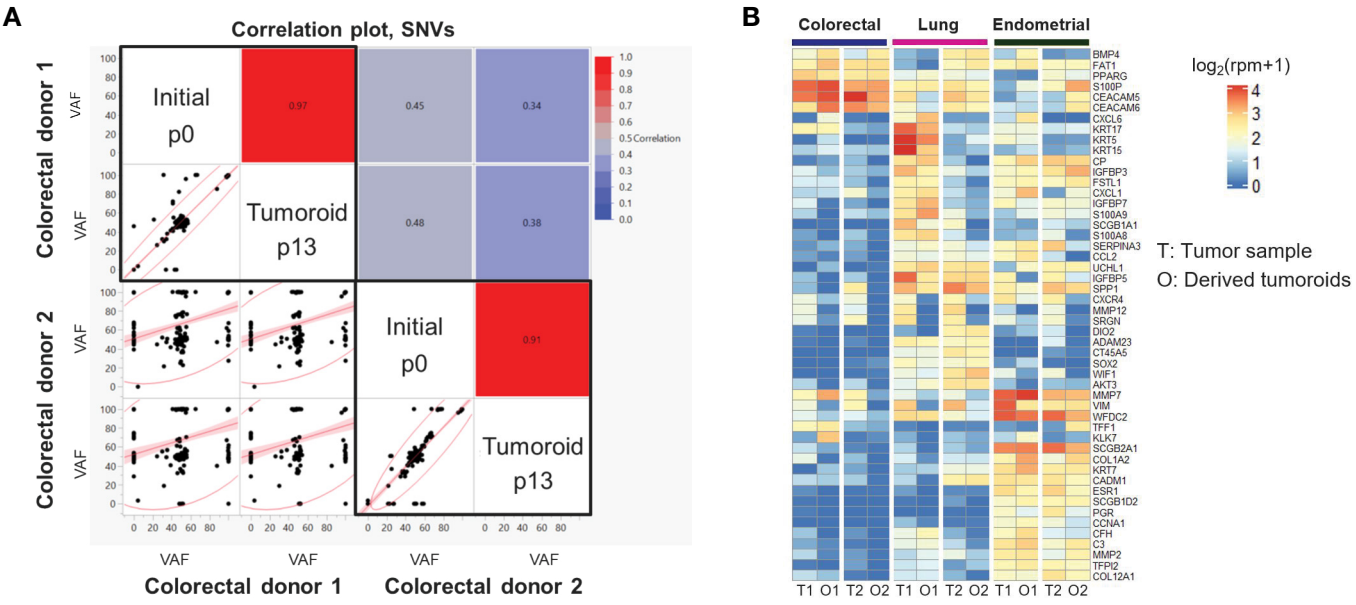

Figure 4. Characterization of tumoroids during culture and expansion. Mutational characterization of established tumoroid lines. **(A)** Comparing variant allele frequency (VAF) of single-nucleotide variants (SNVs) detected in initial samples and established tumoroid lines. **(B)** Heat map comparing gene expression levels for cancer-related genes in derived tumoroids against the original tumor sample for 3 different cancer indications (rpm: reads per million).

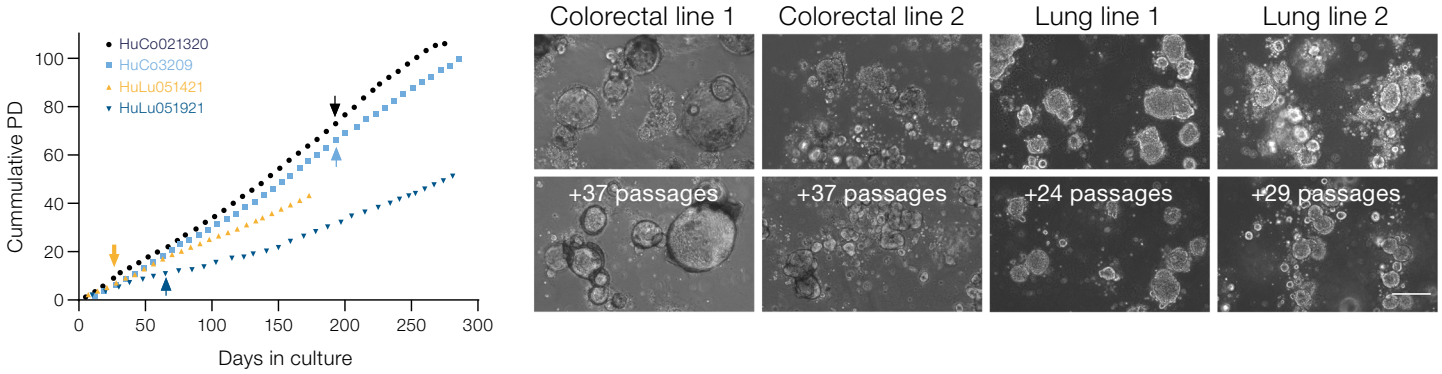

Figure 5. Stability of tumoroid growth rate and morphology over time. Tumoroid growth, measured as population doublings (PD), was monitored over time, and representative images were taken prior to each passage (scale bar = 200 µm).

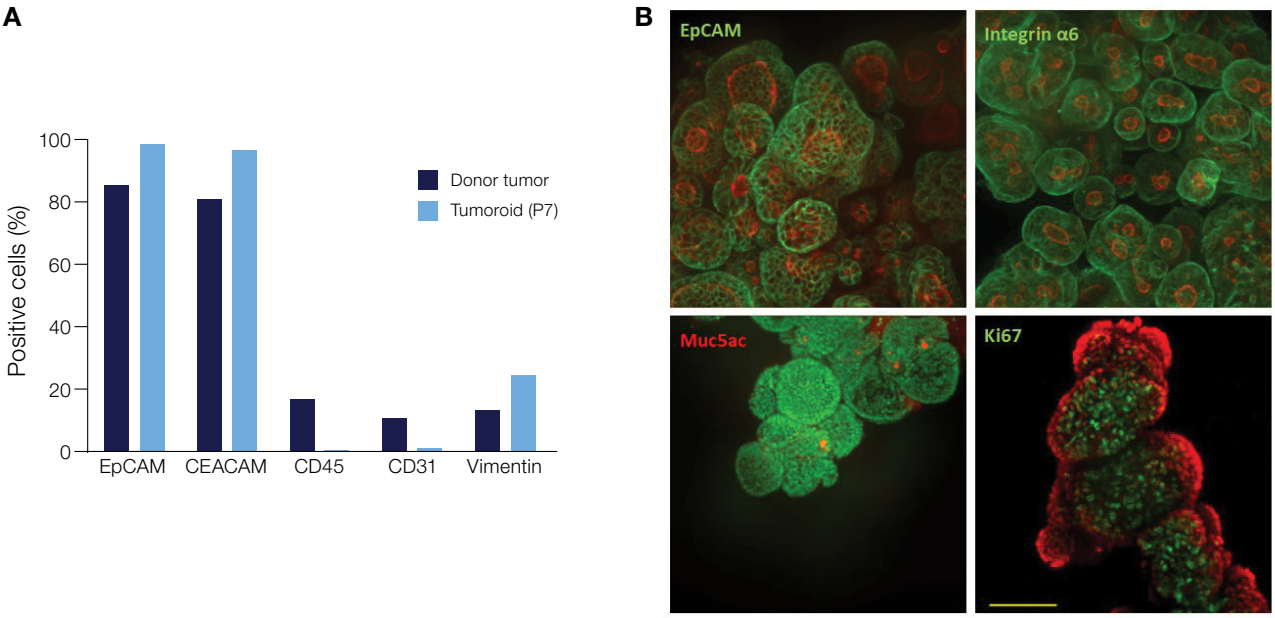

Figure 6. Protein expression analysis and staining of tumoroids. (A) Analysis of protein expression of donor tumor and long-term propagated tumoroids by flow cytometry. **(B)** Intact tumoroids were stained for cancer-related markers (EpCAM, Integrin α6, and Muc5ac) and cell proliferation marker (Ki67). Images were captured using Thermo Scientific™ CellInsight™ CX7 LZR High-Content Screening Platform (scale bar = 100 µm).

Tips and tricks

- Ensure that high-quality RNA is used for the Ion AmpliSeq™ Transcriptome Human Gene Expression Kit. RNA quality can be assessed using an Invitrogen™ Qubit™ RNA IQ Assay. This can be paired with Invitrogen™ Qubit™ RNA High Sensitivity (HS), Broad Range (BR), and Extended Range (XR) Assay Kits for accurate and precise RNA quantification.
- For flow cytometric analysis, ensure cells are in a single-cell suspension. See the **user guide** for additional information on how to further dissociate your tumoroids without significantly impacting cell viability.

Product highlights

Ion Torrent™ Oncomine™ Comprehensive Assay v3—This assay is a targeted, next-generation sequencing (NGS) assay that enables detection of relevant SNVs, CNVs, gene fusions, and indels from 161 unique genes. It can be used for initial characterization and SNV-based fingerprinting of tumoroid cultures.

Ion AmpliSeq™ Transcriptome Human Gene Expression Kit—The Ion AmpliSeq Transcriptome Human Gene Expression panel enables simultaneous measurement of expression levels of over 20,000 human RefSeq genes in a single assay. This helps to assess correlation between the transcriptome profiles of tumoroid lines in long-term culture.

Services

Have our research services team characterize your tumoroids. Characterization services can ensure the transcriptional profile is maintained after adaptation to OncoPro Tumoroid Culture Medium, and confirm quality and lack of contamination. Some characterization services included are:

- Gene expression analysis
- Mycoplasma testing
- Targeted mutation profiling with Oncomine™ Solutions

Contact our team to see all our characterization services offerings by visiting **thermofisher.com/characterizationservices**.

Engineer: Modify tumoroids using electroporation or lentiviral transduction

To understand the role of cancer-driving genes or to engineer tumoroids to express markers for detection in downstream assays, it may be required to perform overexpression or knock down of genes in the tumoroid cells. For transient transfection, electroporation can be performed. For stable pool selection, methods such as electroporation or lentiviral transduction are recommended. These techniques enhance the efficiency of genetic engineering and expedite the process of generating a stable pool (Figure 7). Following engineering, expression of proteins can be assessed using flow cytometry or western blot.

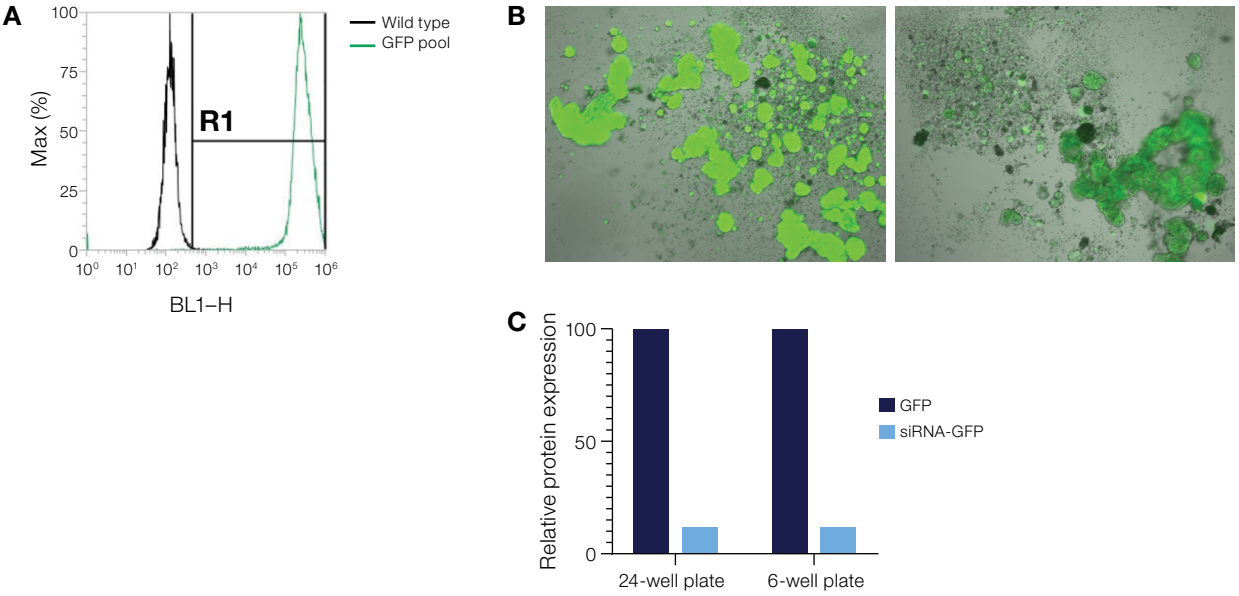

Figure 7. Establishing a stable selection pool of a tumoroid line. (A) Assessment of GFP expression in stable selection pool of a tumoroid line transduced with GFP using lentivirus. Analysis was performed using an Invitrogen™ Attune™ Flow Cytometer. **(B)** GFP knockdown in the line generated in panel A using siRNA. Transfection was performed using the Invitrogen™ Neon™ Transfection System and imaged on an EVOS M7000 Imaging System (left: untransfected cells, right: transfected cells). **(C)** Approximately 90% knockdown of GFP was observed in small scale (24-well plate) as well as large scale (6-well plate) transfections. GFP expression was analyzed on an Attune NxT Flow Cytometer, and data were plotted using GraphPad Prism™ Software 9.0.

Tips and tricks

- Transfect tumoroids immediately after passaging. Small clumps better enable efficient delivery of payloads.
- Do not perform single-cell cloning for selection as it may cause loss of cell heterogeneity within the tumoroid culture.

Product highlights

Invitrogen™ Neon™ NxT Transfection System—When working with a new cell type, it is important to optimize payload delivery conditions. The Neon NxT Transfection System comes with predesigned optimization parameters to help determine the best conditions for delivering payload to a line. It also helps to perform transfection in a wide range of plate formats, from 96-well plates to 100 mm dishes.

Gibco™ CTS™ LV-MAX™ Transfection Kit—Lentiviral (LV) vectors offer unique advantages over other gene delivery systems, especially with the ability to integrate transgenes into the genome of both dividing and nondividing cells. The CTS LV-MAX Transfection Kit is optimized for delivery of multiple DNA plasmids into mammalian cells. Moreover, all components of the kit are produced under CGMP manufacturing conditions to enable its use in cell therapy research.

Services

Our team of scientists can engineer your patient-derived tumoroid lines to express fluorescent proteins, like GFP, or incorporate reporter genes, like the luciferase gene, to use in downstream assays.

Contact your sales representative to learn more, or send us an inquiry at **thermofisher.com/tumoroidservices**.

Assay: Leverage tumoroid models for your unique research aim

Disease-relevant assays, including drug screening, can be performed on both engineered or non-engineered tumoroid lines, and the results can be compared to those of immortalized cancer lines. Tumoroid lines can also be used in assessing response to immune cell therapies, understanding the impact of specific mutations or genes on cell proliferation, developing precision therapies, and in other assays. Reagents designed to measure cell viability, proliferation, and death can be used for high-throughput evaluation of these processes (Figures 8 and 9). For example, Invitrogen™ CellEvent™ Caspase-3/7 reagents can be used with live tumor cells to quantify apoptosis in response to chemotherapeutic drugs or other potential therapeutics. If desired, assays can be multiplexed to gather more information on the effects of chemotherapeutic drugs on tumoroids.

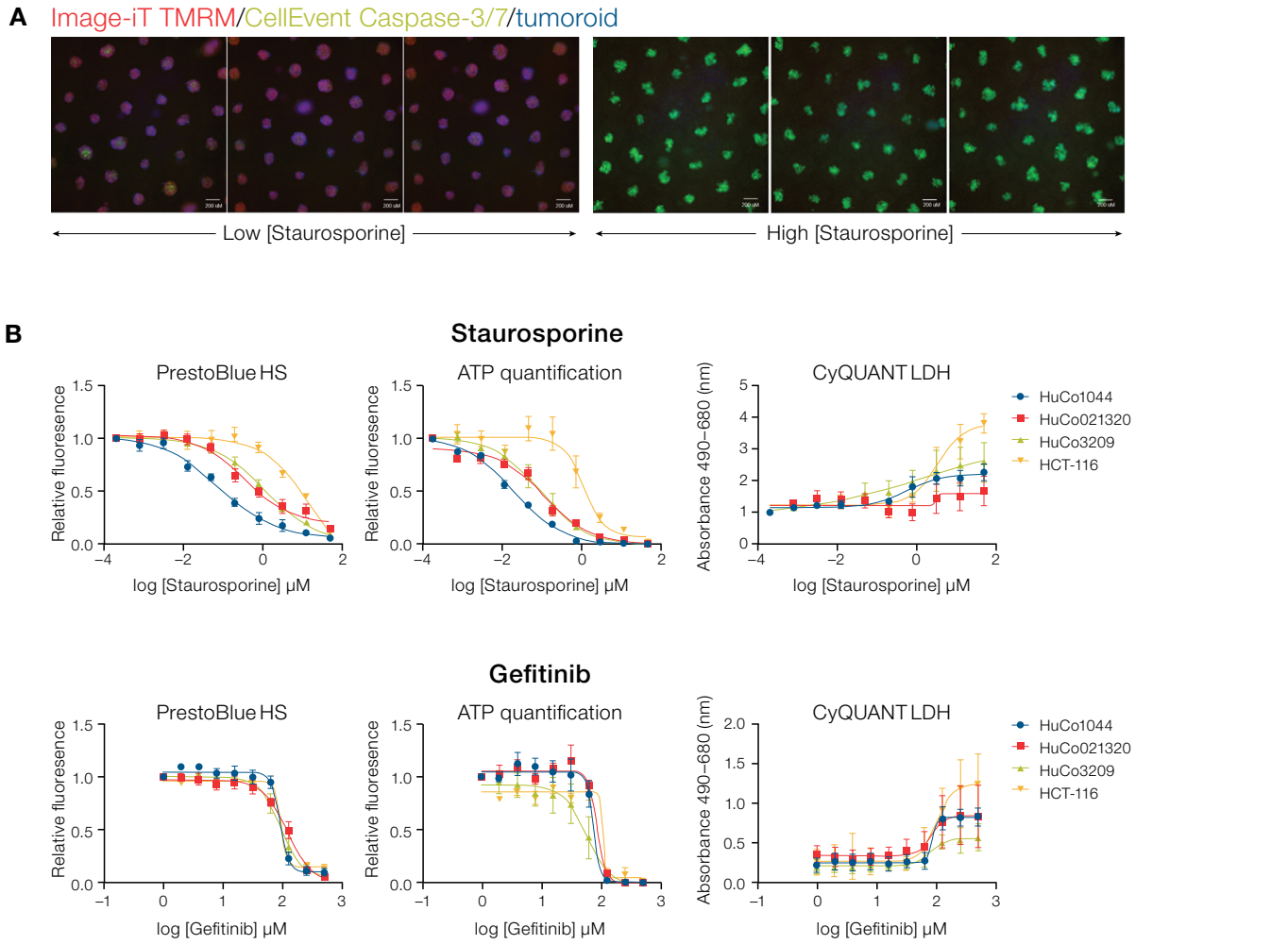

Figure 8. Cell health assessment of tumoroids. (A) Assessment of tumoroids treated with staurosporine using multiplex high-content imaging. Tumoroids were prestained with Invitrogen™ Hoechst 33342 (nuclear marker), Image-iT™ TMRM Reagent (indicator of mitochondrial membrane potential), and CellEvent Caspase 3/7 detection reagent (indicator of apoptosis induction) prior to staurosporine addition. Images were captured using the CellInsight CX7 LZR High-Content Analysis Platform (scale bar = 200 µm). **(B)** Assessment of tumoroids (HuCo1044, HuCo021320, HuCo3209) and an immortalized cancer cell line (HCT-116) after staurosporine or gefitinib treatment using a Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader.

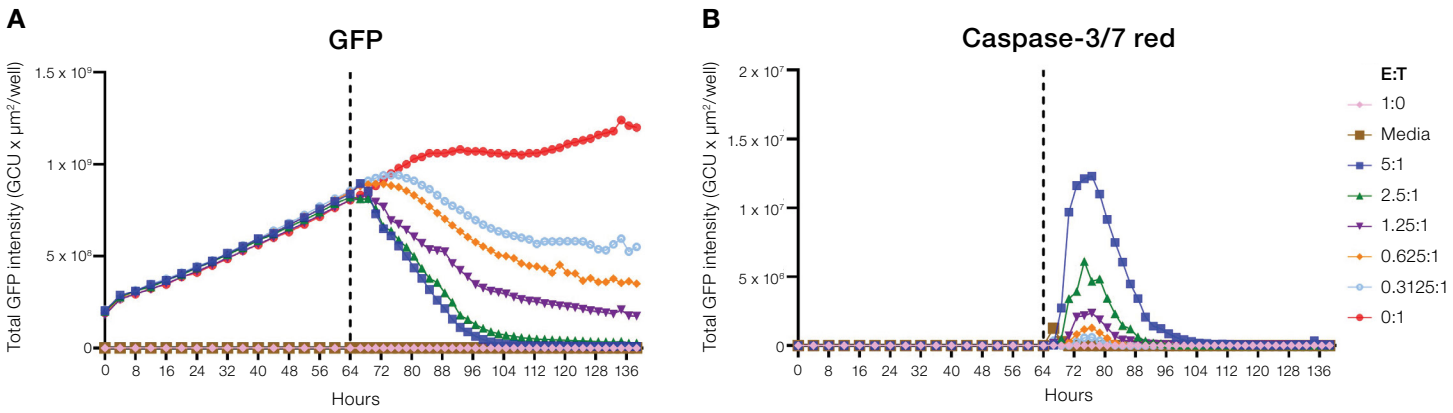

Figure 9. Assessment of immune cell–mediated killing of tumoroid cells. A GFP-overexpressing tumoroid line was preincubated with Invitrogen™ CellEvent™ Caspase 3/7 red reagent and then co-cultured with NK cells in the specified ratios (E:T represents the effector:target ratio). After co-culture initiation at 64 hours, **(A)** cell death (reduction in GFP signal) and **(B)** apoptosis induction (increase in caspase-3/7 red signal) were measured.

Tips and tricks

- Cell health can be assessed over time using a high-content analysis (HCA) instrument with an onstage incubator, such as the CellInsight CX7 LZR HCA Platform.
- By choosing appropriate light filters, cell health assays can be multiplexed to gain a holistic understanding of cell health and behavior.
- For best results, incubate tumoroids with Invitrogen™ PrestoBlue™ HS Cell Viability Reagent overnight prior to taking readings.
- Tumoroid cells remain viable when exposed to PrestoBlue HS Cell Viability Reagent. The reagent can be washed out of the medium for further culture or multiplexed analysis (e.g., fixation and immunofluorescence imaging).

Product highlights

Invitrogen™ PrestoBlue™ HS Cell Viability Reagent—The reagent is a ready-to-use resazurin-based solution that functions as a cell health indicator by using the reducing power of living cells to quantitatively measure viability. Viability measurements can be obtained using a plate reader. Following measurements, the PrestoBlue solution can be removed and tumoroid cells can be propagated further.

Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader—This multimode plate reader enables high-throughput readouts for various assays, in single-plex and multiplex formats. It can measure absorbance, fluorescence intensity, luminescence, AlphaScreen™ assays, and time-resolved fluorescence. Depending on the assay, this system can be used to perform a time-lapse experiment.

Thermo Scientific™ CellInsight™ CX7 LZR High-Content Analysis Platform—This platform is an automated cellular imaging and analysis system that enables high-content imaging of tumoroids for various cell health assays. It has a confocal imaging mode and Z-stacking option that shows which tumoroids of interest will be in focus and have minimal background for fluorescence-based analyses.

Services

Our research services team can support your work by developing assays that include drug screening, immuno-oncology killing assays, and more.

Ordering information

Product	Cat. No.
Source	
OncoPro Tumoroid Cell Lines	Submit inquiry
Hibernate-A Medium	A1247501
Collagenase Type IV	17104019
Hanks' Balanced Salt Solution, calcium, magnesium	14025092
Hanks' Balanced Salt Solution, no calcium, no magnesium	14175095
TrypLE Express Enzyme	12605010
Culture	
OncoPro Tumoroid Culture Medium Kit	A5701201
Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix	A1413201
Nunc Non-treated Flasks	156800
Nunc Non-treated Multidishes	150239
Nunc 15 mL and 50 mL Conical Sterile Polypropylene Centrifuge Tubes	339653
StemPro Accutase Cell Dissociation Reagent	A1110501
Recovery Cell Culture Freezing Medium	12648010
DPBS, no calcium, no magnesium	14190144
SoftFit-L Filtered Pipette Tips in Hinged Racks	2779-HR
Characterize	
EVOS M7000 Imaging System	AMF7000
Oncomine Comprehensive Assay v3	A36111
Ion AmpliSeq Transcriptome Human Gene Expression Kit	A26325
Attune NxT Flow Cytometer	A24864
BlockAid Blocking Solution	B10710
Alpaca Nano secondary antibody	SA5-10328
Ion Chef Instrument	4484177
Ion GeneStudio S5 Prime System	A38411
Engineer	
Neon NxT Electroporation System	NEON1
iBlot 3 Western Blot Transfer Device	A56727
iBright CL1500 Imaging System	A44114
Attune NxT Flow Cytometer	A24864
CTS LV-MAX Lentiviral Production System	A35684
Silencer GFP siRNA	AM4626
Assay	
Human Plasma-Like Medium (HPLM)	A4899101
PrestoBlue HS Cell Viability Reagent	P50201
Varioskan LUX Multimode Microplate Reader	VLBL00GD2
CellEvent Caspase-3/7 Green ReadyProbes Reagent	R37111
CellInsight CX7 LZR Pro High-Content Screening Platform	HCSDCX7LZRPRO
SYTOX Deep Red Nucleic Acid Stain, for fixed/dead cells	S11381
Image-iT TMRM Reagent (mitochondrial membrane potential indicator)	I34361
CyQUANT LDH Cytotoxicity Assay	C20300
Nunc F96 MicroWell Black Polystyrene Plate	237105

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