Isolation of patient-derived primary cancer cells

PromoCell®

Application note

Tumors consist of a heterogeneous mix of multiple interacting cell types organized in a complex hierarchy. Only a small subpopulation of the tumor cells are cancer stem cells (CSCs) capable of driving progression and ultimately the dissemination of the malignancy. The largest proportion of the cells contained in most tumors are non-tumorigenic, differentiated cells and benign cancer-associated cells such as cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs) and stromal cells.

Despite the unique cellular features of CSCs – e.g. tumorigenicity, unlimited proliferation potential, self-renewal and resistance to cytotoxic drugs – the complex organization of tumors complicates the purification and characterization of the causative subpopulation of cancer driving cells.

The lack of specific markers as well as selective *in vitro* culture methods add to the difficulties.

Traditional culture systems for cancer cells based on classical media formulations lack specificity for CSCs. These media predominately support the proliferation of benign cells, e.g., stromal cells, or differentiated (non-tumorigenic) cancer cells, thus leading to a gradual loss of the original CSC population. Only cells isolated from the most malignant types of tumors in these media, from which most traditional cancer cell lines have been established, have been successful. These cell lines, however, are model systems with a long history of selection and adaption in various ill-defined *in vitro* culture conditions and do not comprehensively reflect the behavior of primary tumors [1].

Mouse models have been developed for establishing cultures from tumors with a lower degree of malignancy, since direct *in vitro* isolation was not effective. After several rounds of serial *in vivo* transplantation of tumor tissue in severely immune-compromised mice, the cells of the primary tumor eventually develop into a stable tumor cell population. However, only a small fraction of these tumor cells is stable under traditional *in vitro* culture conditions. Most can only be maintained by serial *in vivo* transplantation in the mouse. These techniques are expensive, time-consuming and elaborate, and – most importantly – they induce major changes in the initial primary tumor cells inherent in a serial selection process in rodents. Consequently, direct *in vitro* isolation of patient-specific primary tumor cells in an unbiased defined culture environment is highly preferable.

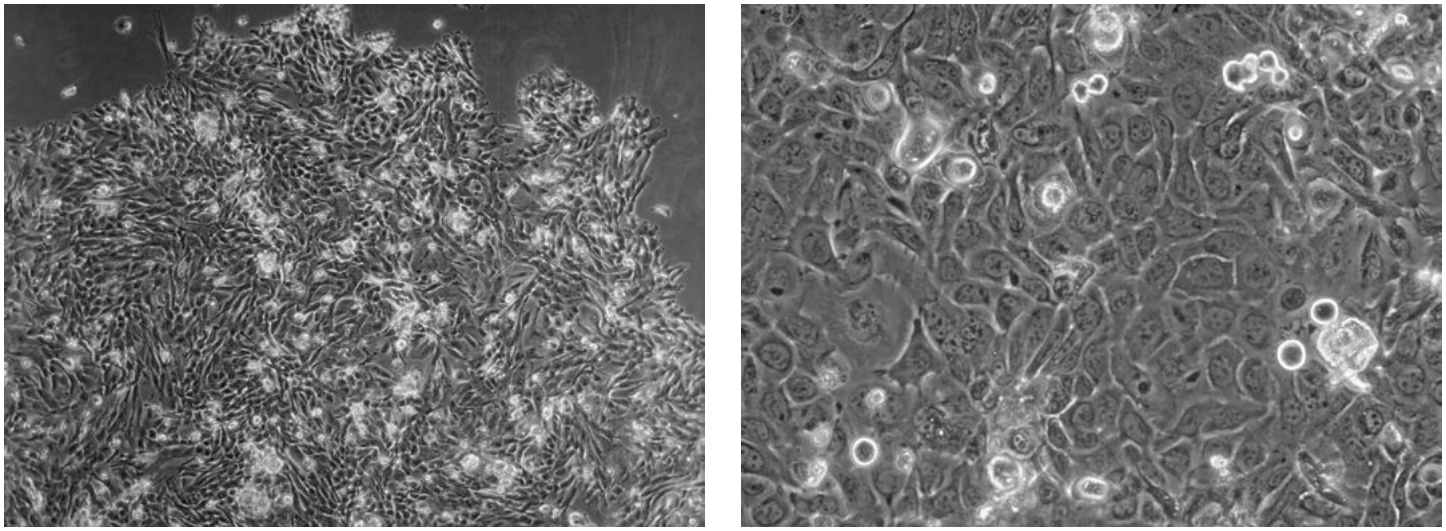

Fig. 1: Primary culture derived from a squamous cell carcinoma, isolated with the Primary Cancer Culture System (PCCS). Left: *The primary culture was obtained after 4 weeks as compact and heterogeneous adherent colonies formed by different types of epithelial-like cells. The culture proliferated in the Primary Cancer Cell Medium D-ACF with a population doubling time of approximately 7 days and could be serially passaged with no signs of growth inhibition.* ***Right:*** *Greater magnification reveals the cellular heterogeneity within the colony. See Tab. 1 for mutational analysis data on this primary culture.*

The principle of the Primary Cancer Culture System (PCCS)

This advanced culture system, consisting of the Primary Cancer Cell Medium D-ACF and the NCCD-Reagent, was designed to be the first universally applicable, cost-effective solution for *in vitro* isolation and long-term primary cultures of human CSCs, e.g., from patient tumor samples or patient-derived xenografts (PDX).

The PCCS is a functional medium, designed to support specifically the aberrant metabolic traits of malignant cells. Since on the cellular level CSC-specific traits represent the only selection criterion, the cell diversity of the cancerous subpopulations of the original tumor is preserved.

With regard to cancer cell primary isolation, traditional tumor cell media generally support the growth of benign cells, e.g., tumor stroma, CAFs and TAMs, but do not sufficiently support the cancer cell subpopulations that drive the progression of tumors *in vivo*. The vast majority of these *in vitro* primary cultures are therefore transient and exhibit a gradual loss of the original CSCs that cause the disease.

In contrast, the Primary Cancer Culture System makes it possible to reliably deplete benign cells from the culture due to the selective support of CSCs. This process usually takes a few to several weeks depending on the intrinsic growth rate of the CSC population within the sample.

Since malignancy itself serves as the sole functional selection criterion, the culture system is applicable to all types and entities of CSCs, regardless of their origin and the stage of the tumor. Provisional enrichment techniques, e.g., cell sorting while relying on unproven markers, are therefore obsolete.

The selection process dispenses with the use of cytotoxic agents in a defined and animal-free culture environment. One bottle of medium is typically sufficient for 3–5 primary isolations.

The Primary Cancer Culture System can also be used for other applications, e.g., enriching CSC subpopulation(s) in established cell lines or depleting of stromal cells and other non-cancerous cells from established primary cancer cell cultures to identify CSC biomarkers.

Gene	Analyzed region	Transcript variant	DNA mutation	Protein mutation	Mutational load#
AKT1	Exon 4	ENST00000349310	-	-	-
ALK	Exon 22, 23, 25	ENST00000389048	-	-	-
BRAF	Exon 11, 15	ENST00000288602	-	-	-
CTNNB1	Exon 3	ENST00000396183	-	-	-
DDR2	Exon 5, 8, 12, 13, 14, 15, 17	ENST00000367921	-	-	-
EGFR	Exon 12, 18, 19, 20, 21	ENST00000275493	-	-	-
ERBB2	Exon 21, 22, 23	ENST00000406381	-	-	-
ERBB4	Exon 3, 4, 6, 7, 8, 9, 15, 23	ENST00000342788	-	-	-
FBXW7	Exon 5, 8, 9, 10, 11	ENST00000281708	-	-	-
FGFR1	Exon 4, 7	ENST00000447712	-	-	-
FGFR2	Exon 7, 9, 12	ENST00000358487	-	-	-
FGFR3	Exon 7, 9, 14, 16, 18	ENST00000340107	-	-	-
KRAS	Exon 2, 3, 4	ENST00000256078	-	-	-
MAP2K1	Exon 2	ENST00000307102	-	-	-
MET	Exon 2, 14, 16, 19	ENST00000318493	-	-	-
NOTCH1	Exon 26, 27	ENST00000277541	-	-	-
NRAS	Exon 2, 3, 4	ENST00000369535	-	-	-
PIK3CA	Exon 10, 14, 21	ENST00000263967	c.1633G>A	p.(E545K)	26%

PTEN	Exon 1, 3, 6, 7, 8	ENST00000371953	-	-	-
SMAD4	Exon 3, 5, 6, 8, 9, 10, 11, 12	ENST00000342988	-	-	-
STK11	Exon 1, 4, 5, 6, 8	ENST00000326873	-	-	-
TP53	Exon 2, 4, 5, 6, 7, 8, 10	ENST00000269305	c.298C>T c.586C>T	p.(Q100*) p.(R196*)	33% 64%

Tab. 1: Mutational analysis of the squamous cell carcinoma primary isolate depicted in Fig. 1. *The tumor panel test detected three hotspot mutations: one in the pik3ca gene and two in tp53. The high mutational load is indicative of a selectively enriched culture of malignant cells, while the differing percentages of the individual mutations suggest the maintenance of cancer cell subpopulation heterogeneity in vitro. #Mutational load = percentage of mutated transcripts/total transcripts of the respective transcript variant.*

Cancer cell isolation from tumor tissue samples

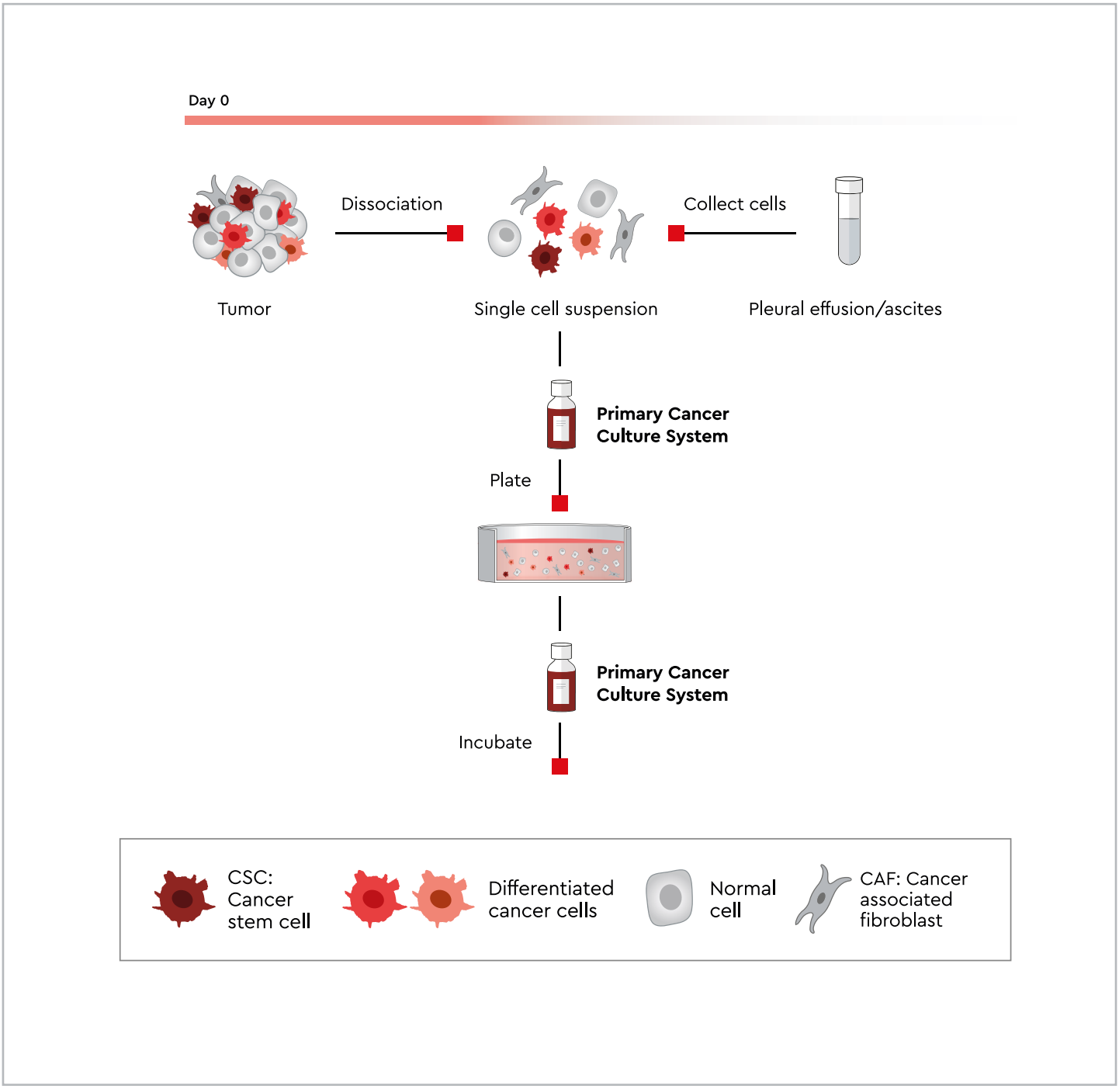

Fig. 2: Tumor cell isolation from biopsy using PCCS (C-28081)

Separation of adherent cancer cells from primary aggregates

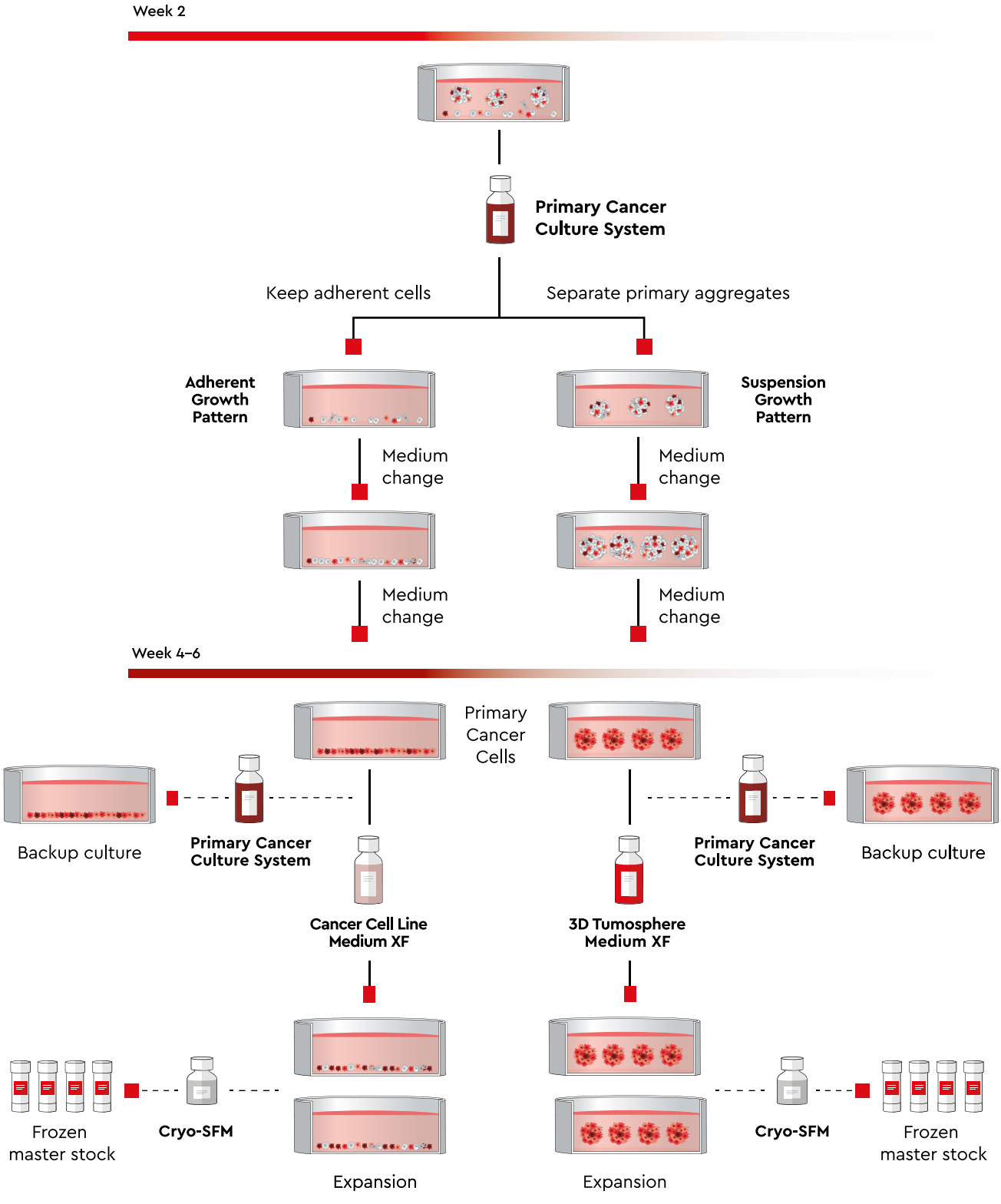

Fig. 3: Cancer cell culture expansion and separation of tumorspheres from adherent cells.

Protocol for establishment of primary cancer cell lines

Depending on the tissue quality, type and malignancy stage of the tumor sample, obtaining a homogeneous primary culture may require 4–8 weeks.

I. Cancer cell isolation from tumor tissue samples

Materials

- Fresh tumor tissue (0. 5–3 grams; ≥ 1 gram is optimal)
- Hanks Balanced Salt Solution (HBSS) with Ca^{2+}/Mg^{2+} without Phenol Red
- Primary Cancer Culture System (C-28081)*
  *consists of the Primary Cancer Cell Medium D-ACF and 2 ml of NCCD-Reagent (C-43080; also available separately)
  RPMI, MEMalpha or a comparable standard TC Basal Medium
- Gentamicin (50 mg/ml stock)
- Phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+} (C-40232)
- Accumax (e.g. Sigma #A7089) – tissue digestion/isolation
- Accutase (C-41310) – passage/subcultivation of established culture
- Scalpel/forceps/scissors
- Cell strainers of descending size down to 40 µm (e.g. 400/100/40 µm)
- Tilt-roll-shaker, rotary mixer or comparable
- Tissue culture flasks and dishes

Use aseptic techniques and a laminar flow bench.

1

NCCD Treatment of the Plasticware (day 0 or earlier)

The use of the NCCD-Reagent provided with the Primary Cancer Culture system is indispensable for successful isolation and maintenance of cancer cells. Dilute the thawed NCCD-Reagent stock solution 1:20 with PBS. Use 100 µl/cm^2 of culture surface to treat the tissue culture vessel with the diluted NCCD-Reagent and leave the closed vessel for at least one hour at room temperature. Make sure that the NCDD covers the entire vessel surface. Aspirate the NCCD solution just before seeding the cells

Note: Unless used immediately, the sealed vessel containing the NCCD-Reagent may be stored for up to three months at 2–8°C for later use. Diluted NCCD-Reagent solution may be stored for up to four weeks at 2–8°C protected from light.

2

Wash and weigh the tumor tissue

Remove visible residues of healthy tissue from the tumor. Place the tumor sample in a tube and wash twice with a generous amount of PBS and vigorous shaking. Then weigh the tumor tissue in a pre-tared sterile petri dish.

Note: The tumor tissue should be as fresh as possible and stored in HBSS at 2–8 °C immediately after surgical removal. Tissue up to six hours old is optimal for isolation purposes. However, successful isolations have been accomplished from tumor samples as old as 24 hours. Keep in mind that recently applied chemical or radiation therapy may severely affect the isolation results.

3

Homogenize the tumor tissue

Place the washed tumor sample on the lid of a petri dish. Add a small volume (1–2 ml) of Primary Cancer Cell Medium D-ACF to the tumor tissue and dissect it into small pieces of approximately 1 mm^3 using a scalpel. Avoid attrition of the tissue.

4

Wash the homogenized tumor tissue

Transfer the homogenized tumor tissue to a 50 ml tube using forceps. Add 10x the volume (w/v) of PBS and then invert the tube 5–10 times to remove residual blood and debris associated with the pieces of tumor tissue. Let the tissue pieces settle for two minutes and then aspirate the supernatant. Repeat if there is still a lot of blood/debris observable. Remove as much as possible of the PBS without loosing the tissue.

Note: If there is floating homogenized tissue, use a suitable sieve or cell strainer, for separating the washed, homogenized tissue from the washing buffer.

5

Perform the enzymatic digest of the tumor tissue

Resuspend the tissue pellet in Accumax solution at a concentration of 20 ml per gram of tumor tissue. Incubate at room temperature (20-25°C) with gentle but constant mixing, e.g. by a tilt-roll mixer at 40-50 rpm. Addionally invert the tube manually every 5 minutes. Digest until the solution becomes distinctly turbid. Depending on the type of tissue, this is typically the case after approximately 30–45 minutes. A 35-minute incubation is a good starting point.

Note: Do not digest the tissue longer than necessary and never digest for longer than 45 minutes since this may significantly compromise cell viability. Always perform the digestion reaction at room temperature and consult the Accumax manual for instructions on proper storage and handling.

6

Remove tissue residues from the sample

Let the remaining tissue pieces settle down for 2 minutes. In order to obtain a single-cell suspension, progressively filter the turbid supernatant using cell strainers of descending pore size down to 40 µm, e.g. 400 µm → 100 µm → 40 µm.

Note: Discard the remaining tissue pieces.

7

Dilute the sample with medium

Dilute the single-cell suspension at least 1:4 with RPMI or a comparable Basal Medium. Use a higher dilution ratio if the solution is still viscous.

8

Obtain the isolated single cells

Pellet the cell suspension for 10 minutes at 240 x g at room temperature and carefully aspirate the supernatant without disturbing the cell pellet.

9

Determine the number of viable nucleated cells

Gently resuspend the cell pellet in 5 ml of Primary Cancer Cell Medium D-ACF. Combine all cell pellets in 5 ml of medium in case your sample was divided into several tubes during the dilution step (7). Determine the number of viable nucleated cells using an appropriate method.

Note: In case of cell clumps, which cannot be resuspended, filter the cell suspension once more through a 40 µm cell strainer before counting. The expected yield is 1–3 million viable nucleated cells per gram of tumor tissue.

If it is not possible for any reason to determine the viable nucleated cell count in the primary isolate, continue with step 10 and refer to the Note in step 11. Keep in mind that omitting cell counting may lead to suboptimal seeding densities which may strongly impede the isolation efficiency.

10

Wash the cells

Pellet the cell suspension for 10 minutes at 240 x g at room temperature and carefully aspirate the supernatant without disturbing the cell pellet. Finally, resuspend the cell pellet in 1 ml of Primary Cancer Cell Medium D-ACF.

11

Plate the cells

Plate 100.000 to 300.000 viable nucleated cells per cm^2 in the prepared NCCD- treated tissue culture vessel(s). Use approximately 300 µl of medium per cm^2 for vessels ≤ 25 cm^2 of culture surface and approximately 130 µl medium per cm^2 for > 25 cm^2. Add 50 µg/ml of Gentamicin to the final volume and incubate at 37°C with 5% CO_2.

Example: Plate 1–3 million nucleated viable cells per well of a 6-well plate using 3 ml of medium. Plate 2.5–7.5 million nucleated viable cells per T-25 flask using 5 ml of medium. lation efficiency.

Note: If the viable nucleated cell count was not determined in step 9, then plate the primary isolate from up to 2 grams of tumor tissue in 1–2 wells of a 6-well plate using 3 ml of Primary Cancer Cell Medium D-ACF per well.

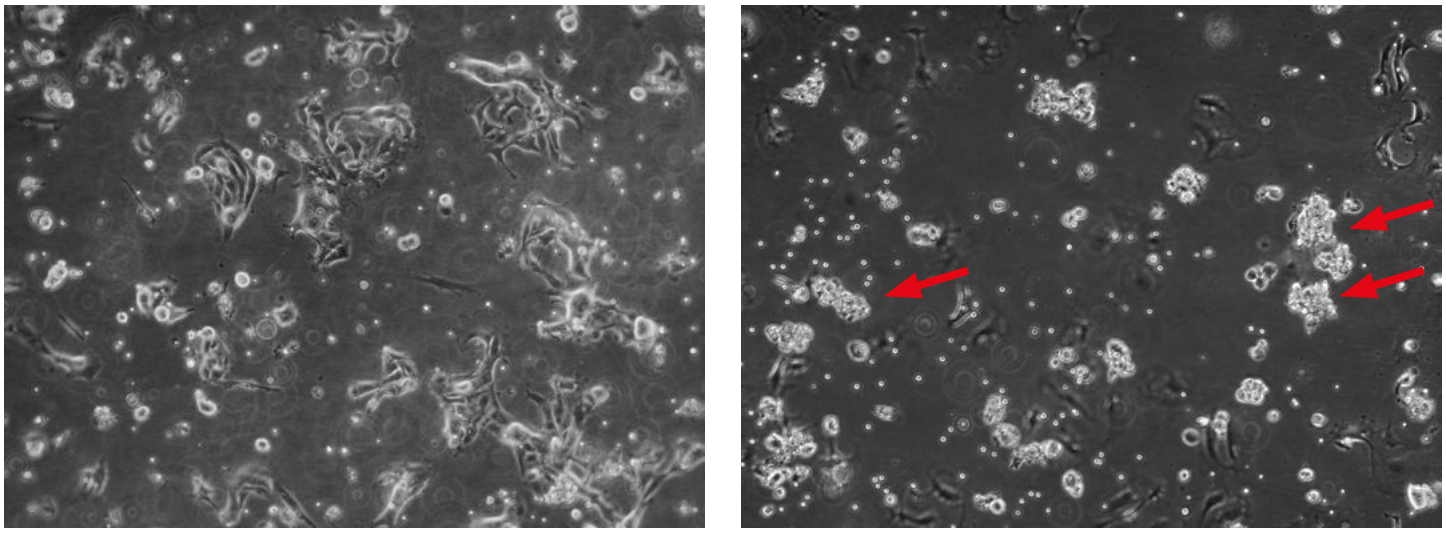

Fig. 4: General appearance of primary cancer cell cultures in the Primary Cancer Cell Medium D-ACF in early stages, in this example with lung squamous cell carcinoma cells. Left: *Culture on day 2 after initial plating: a mix of residual erythrocytes, fibroblast- and epithelial-like adherent cells as well as floating single suspension cells can be observed.* ***Right:*** *On day 11, the formation of floating multicellular cell aggregates is already prominent (red arrows). The culture was used for primary aggregate separation on day 13 and was additionally cultured in a new flask parallel to the original sample containing the remaining adherent cell fraction (see Fig. 3 and protocol step 14).*

II. CSCs selection and expansion

12

Initiate of the primary tumor cell culture (day 0)

Incubate the culture for a total of 10–14 days to let the primary tumor cell culture begin but proceed with step two on day six after plating (see step 13).

Note: Typically, adherent and non-adherent cells as well as formation of multicellular primary suspension aggregates can be observed during the first two weeks of culture.

13

Add fresh medium (day 6)

On days 5–7, add an additional volume of the initial culture volume of fresh medium (without antibiotics) to the cells. Do not change the medium; simply add more fresh medium. Continue incubation until the culture reaches the stage described in step 14.

Example: : For an existing culture with a volume of 5 ml of medium, add another 5 ml of fresh medium. The resulting total culture volume is then 10 ml.

Note: If the medium turns orange-yellow due to high metabolic activity of the isolated cells before day six, the fresh medium should be added sooner. A slightly orange color is noncritical, however. If significant media exhaustion is still observed before the culture is ready for step 14, increasing the total culture volume by adding fresh medium is recommended.

14

Initiate a separate secondary suspension culture (days 10–14)

The primary culture is ready for step 14 as soon as sufficiently large floating multicellular aggregates (i.e. ≥70 µm in diameter) have developed or small aggregates of fewer than 10 cells appear. This is usually the case after 10–14 days. Perform step 14 no later than day 14. Depending on the suspension cell pattern of your primary culture, continue with step 14a or 14b, whatever is more appropriate.

Performing step 14 results in two separate culture vessels: the original primary flask containing the residual adherent cells and a new secondary flask containing the suspension cell fraction (see Fig. 3).

14a

Separation of larger aggregates (≥70 µm or ≥10 µm cells)

14b

Separation of small aggregates and single suspension cells

Collect the used medium containing the small aggregates/single suspension cells in a separate 15 ml conical tube. Wash remaining adherent cells twice with PBS, immediately add an appropriate amount of fresh medium (see below) and return them to the incubator.

Centrifuge the suspension cell sample for 10 minutes at 240 x g at room temperature. Leave 200 µl of the supernatant behind while gently aspirating the spent medium, since the pellet may be quite loose. Resuspend and plate the cells in an appropriate amount of fresh medium (see below) in a separate new culture vessel treated with the NCCD-Reagent.

Recommended media volume: Use approximately 300 µl of medium per cm^2 for vessels with ≤ 25 cm^2 of culture surface and approximately 130 µl medium per cm^2 for > 25 cm^2. Continue incubation of the cultures at 37°C and 5% CO_2.

14c

Add fresh medium

Add another volume of fresh medium to each culture seven days after separating the adherent/suspension cells.

Note: The residual adherent cell fraction in the original primary culture flask can contain stromal cells and other types of unwanted benign cells but may still contain cancer cells. Therefore, closely monitor the culture of the adherent cell fraction for significant sustained proliferation, e.g. colony formation. "Budding" of new aggregates or suspension cells from existing adherent cell clusters may be observed during up to four weeks. Combine newly formed aggregates/cells with the separate secondary suspension culture during the regular medium changing interval (refer to step 15).

Note that separated suspension aggregates may revert to an adherent or intermediate growth pattern in the further course of the isolation process.

15

Change medium during cancer cell selection (every 10–14 days)

After successfully completing step 14, completely replace the medium of all samples every 10–14 days as described in steps 15a-15d. The appropriate media change technique will depend on the growth pattern of the corresponding primary isolate. Methods for each of the possibilities are described in steps 15a-15c. Adherent cultures remain in the same culture vessel until the first passage (see step 7). In the case of suspension cultures, replacing the used vessel with a new one when regularly changing the medium is optional but not mandatory in most cases. Always keep flasks with adherent cells for at least four weeks or until you are absolutely sure they do not contain cells of interest.

Note: Make sure to prevent extensive medium exhaustion (indicated by an orange-yellow color; a slightly orange hue is still acceptable). Isolations in which no viable primary culture has become successfully established within six weeks after initial plating are not promising and can be discarded.

15a

Change medium for adherent cultures

Aspirate the used medium of adherent cells, wash the culture twice with PBS and add an appropriate amount of fresh medium (see below) to the cells. If the spent medium contains significant amounts of suspension cell aggregates or viable single cells, use the passage techniques described in 15b and 15c to recover these cells.

15b

Change medium for large cell aggregates (≥ 70 µm)

Collect the used medium containing the suspension aggregates in a separate 15 ml conical tube. If applicable, wash remaining adherent cells twice with PBS, immediately add an appropriate amount of fresh medium (see below) and return them to the incubator.

Let the tube with the suspension aggregates stand upright for 12 minutes at room temperature for gravity sedimentation of larger cell aggregates. Then gently aspirate the supernatant while leaving 0.5–1 ml behind. Carefully transfer the sedimented aggregates with a serological pipet into a NCCD-treated culture vessel containing an appropriate amount of fresh medium (see below).

Note: In contrast to other sphere culture techniques, disaggregation of the primary cell aggregates is neither recommended nor necessary.

15c

Change medium for small aggregates and single suspension cells

Collect the used medium containing the small aggregates/single suspension cells in a separate 15 ml conical tube. If applicable, wash remaining adherent cells twice with PBS, immediately add an appropriate amount of fresh medium (see below) and return them to the incubator.

Centrifuge the suspension cell sample for 10 minutes at 240 x g at room temperature. Leave 200 µl of the supernatant behind when gently aspirating the spent medium, since the pellet may be quite loose. Resuspend the pellet in fresh medium and use a serological pipette to transfer the cell suspension into a NCCD-treated culture vessel containing an appropriate amount of fresh medium (see below).

Recommended media volume: Use approx. 300 µl of medium per cm^2 for vessels with ≤ 25 cm^2 of culture surface and approx. 130 µl medium per cm^2 for > 25 cm^2. Continue incubation of the cultures at 37°C and 5% CO_2.

15d

Add fresh medium (day 6 after changing the medium)

Add another total culture volume of fresh medium to each culture after another 5–7 days.

Example: For a 5 ml culture, add 5 ml of fresh medium for a total culture volume of 10 ml.

16

Determine the growth pattern of the isolated CSCs (weeks 2–4)

Adherently growing CSCs can typically be identified within 2–4 weeks after plating since they are present as slow-growing colonies. Follow the regular medium changing schedule for adherent cells without passaging the cells until they reach an adequate confluency level (see step 7).

It is more difficult to assess the traits of isolated CSCs that exhibit a suspension growth pattern, in other words as cell aggregates or single cells. Typically, they proliferate very slowly and intrinsically lack morphological information on the level of single cells. It is therefore recommended to wait for the numbers of CSCs to increase sufficiently to permit further characterization and in-depth analysis (see also the Note in step 17). It is highly advisable to keep all of the primary cultures until you have unequivocally identified the growth pattern of your isolated CSCs.

Note: Depending on the properties of the primary tumor and the doubling times of CSCs in vivo [2–4], the selective culture conditions may result in slow growth of the isolated cells with doubling times ranging from a couple of days to several months (near-quiescent state). In addition, the initial cell loss caused by successive depletion of non-cancerous cells from the primary isolate may also slow down expansion of the isolate, especially in the first weeks after initial plating.

In general, primary CSC isolates that exhibit an adherent growth pattern tend to proliferate significantly faster than their counterparts growing in suspension.

17

Determine the growth pattern of the isolated CSCs (weeks 2–4)

After making sure that all unwanted benign cells have been eliminated, you can set up your experiments with the isolated CSCs. Alternatively, the cells may be passaged and expanded further (see step 18 and III) or cryopreserved (see IV).

Note: We strongly recommend always keeping a backup of the stock culture in the Primary Cancer Cell Medium D-ACF in combination with NCCD-treated culture vessels to ensure long-term maintenance of the unaltered CSCs.

18

Passage the tumor cell primary culture

Passaging the cells before they proliferate to a high confluence level is not recommended. Until they do, continue changing the medium as described for step 4.

18a

Passage the suspension cultures

Increase the total culture volume by adding fresh Primary Cancer Cell Medium D-ACF and split it in two fresh NCCD-treated vessels (see below for the recommended amounts of medium). Use gravity sedimentation for the regular medium changes to reduce the amount of debris in the culture.

Note: In contrast to other types of sphere cultures, it is not necessary to disaggregate cell clusters because aggregates of malignant cells propagate autonomously under these culture conditions.

18b

Passage the adherent cells

Prepare new NCCD-treated culture vessels (see step 1). Depending on the overall confluence, perform a 1:1 or 1:2 split of the culture using Accutase (not Accumax). Wash the culture twice with ambient tempered PBS without Ca^{2+}/Mg^{2+} and then incubate the cells for 5–10 minutes with 100 µl/cm2 Accutase at 37°C. After the first 5 minutes of incubation, monitor the detachment process visually. When the cells start to detach, facilitate their complete dislodgement by tapping the flask. Add the same volume of Primary Cancer Cell Medium D-ACF to the detached cells and spin down for 3 minutes at 300 x g at room temperature. Carefully aspirate the supernatant and gently resuspend the cell pellet in an appropriate amount of fresh Primary Cancer Cell Medium D-ACF (see below). Seed the cells into new NCCD-treated vessels and incubate them further at 37°C and 5% CO_2.

Recommended media volume: Use approximately 300 µl of medium per cm^2 for vessels with ≤ 25 cm^2 of culture surface and approximately 130 µl medium per cm^2 for > 25 cm^2. Continue incubation of the cultures at 37°C and 5% CO_2.

18c

Add fresh medium

Add another volume of fresh medium to each flask after 5 – 7 days (or earlier if required) and continue incubating at 37°C and 5% CO_2.

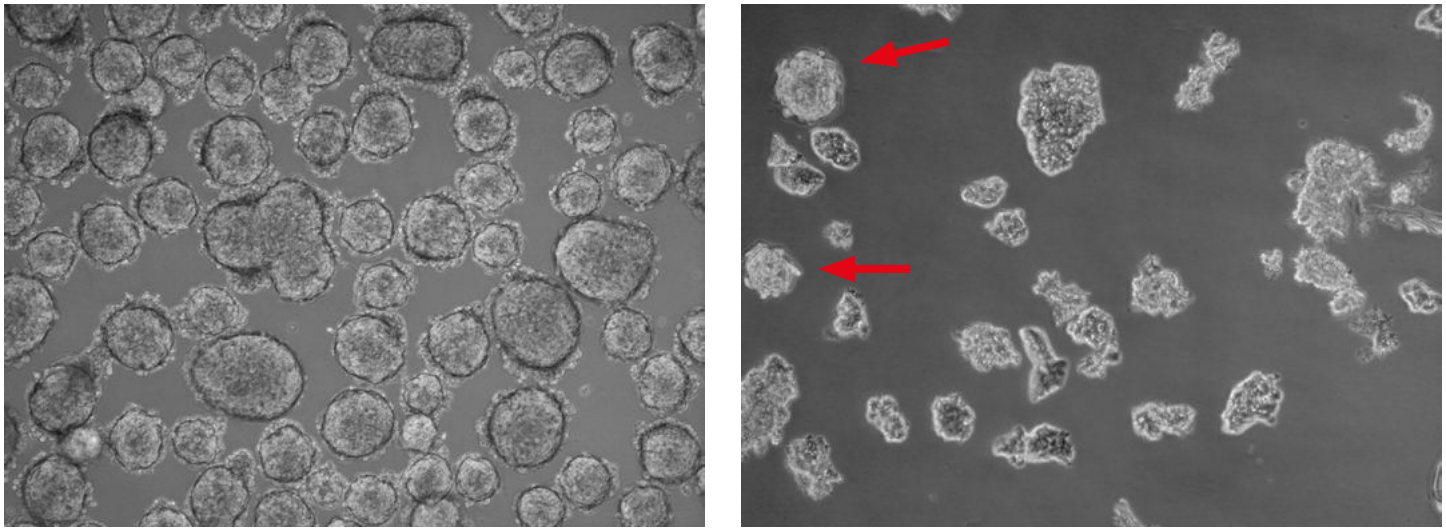

Fig. 5: Primary CSC culture derived from a low-grade small cell lung cancer (SCLC) with the Primary Cancer Culture System. Left: *The primary isolate was obtained after four weeks as a floating sphere-forming culture, which persisted in a near-quiescent state even after six months.* ***Right:*** *Adding extra growth factors elicited significant expansion in the latent sphere culture with a doubling time of 3–4 weeks. Note that some spheres persisted under these modified culture conditions (red arrows), while the larger part of the culture proliferated as floating planar multicellular 2D sheets, which is a prototypical growth pattern for SCLC cells in vitro.*

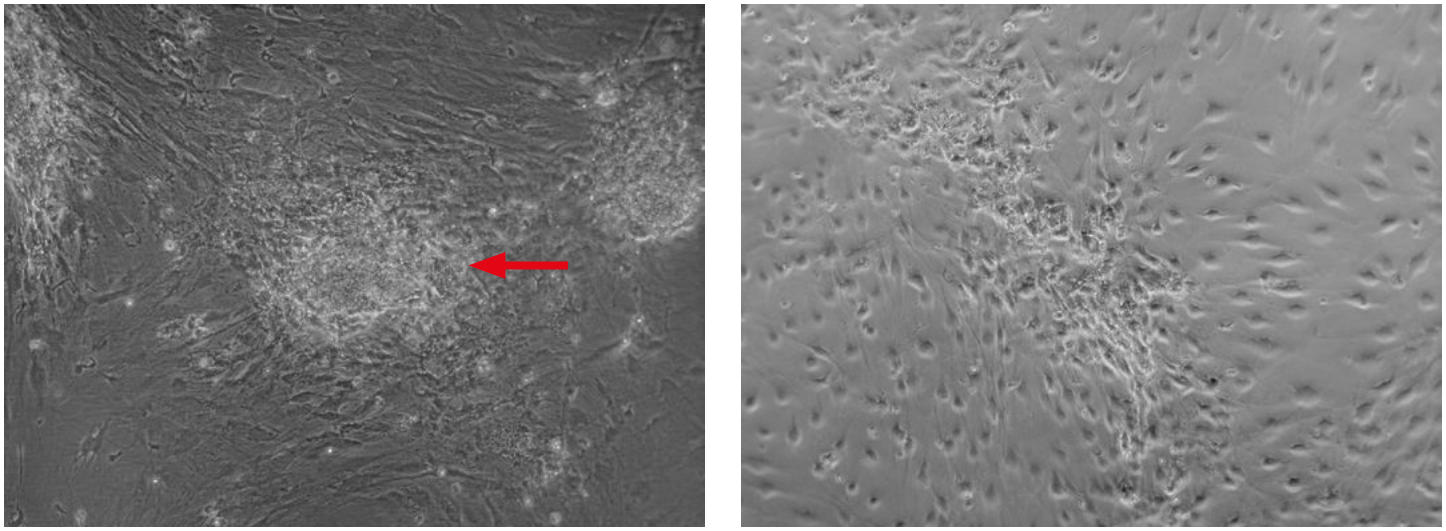

Fig. 6: Primary CSC culture derived from an invasive adenocarcinoma with the Primary Cancer Culture System. Left: *During the first two weeks of the isolation process, the cancer cells appeared as locally restricted, lightly adherent convex cell clusters (red arrow) on top of the stroma layer.* ***Right:*** *After four weeks, highly motile cancer cells began migrating from their original locations to cover the whole culture surface. These cells proliferated as a homogeneous population in the Primary Cancer Cell Medium D-ACF.*

III. CSCs selection and expansion

19

Switch to another culture medium

Use cells in the stage as described in step 18 and passage them into the alternative culture medium as described in step 1a or 1b depending on their culture pattern.

19a

Passage suspension cultures to an alternative growth medium

The 3D Tumorsphere Medium XF (C-28070) may be used as an alternative expansion medium for established non-adherent primary cancer cultures. Collect the used medium containing the suspension aggregates in a separate 15 ml conical tube. If applicable, wash remaining adherent cells twice with PBS, immediately add an appropriate amount of fresh medium and return them to the incubator.
Let the tube with the suspension aggregates stand upright for 12 minutes at room temperature for gravity sedimentation of larger cell aggregates. Then gently aspirate the supernatant while leaving a rest of 0.5–1 ml in the tube. Carefully transfer the sedimented aggregates with a serological pipet into new culture vessels containing an appropriate amount of the alternative culture medium. A 1:2 split ratio is recommended.

19b

Passage adherently growing cells

The Cancer Cell Line Medium XF (C-28077) may be used as an alternative expansion medium for established adherent primary cancer cultures. Prepare new NCCD-treated culture vessels (see step 1). Depending on the overall confluence, perform a 1:1 or 1:2 split of the culture using Accutase (not Accumax). Wash the culture twice with ambient tempered PBS without Ca^{2+}/Mg^{2+} and then incubate the cells for 5–10 minutes with 100 µl/cm² Accutase at 37°C. After the first 5 minutes of incubation, monitor the detachment process visually. When the cells start to detach, facilitate their complete dislodgement by tapping the flask. Add the same volume of complete medium to the detached cells and spin down for 3 minutes at 300 x g at room temperature. Carefully aspirate the supernatant and gently resuspend the cell pellet in an appropriate amount of fresh complete medium. Seed the cells into new NCCD-treated vessels and incubate them further at 37°C and 5% CO_2.

19c

Add fresh medium/perform a medium change

Add another volume of fresh medium or perform a complete medium change using the alternative culture medium as required and continue incubating at 37°C and 5% CO_2.

20

Expand and passage the cells

Expand and passage the cells using the alternative culture medium as required using your established standard tissue culture procedures.

IV. Cryopreservation

For cryopreservation, harvest the isolated cells or cell aggregates as described in section III depending on the growth pattern of the culture. After aspiration of the used culture medium supernatant, gently resuspend the cell pellet or cell aggregates in an appropriate amount of cell-freezing medium and swiftly perform the cryopreservation according to your established standard procedures. For best results, the use of CryoSFM (C-29910) is recommended.

Products

Media	Size	Catalog number
Primary Cancer Culture System consists of		C-28081
Primary Cancer Cell Medium D-ACF	250 ml	C-28080*
Primary Cancer Cell Medium D-ACF SupplementMix	for 250 ml	C-39880*
NCCD-Reagent	2 ml	C-43080
3D Tumorsphere Medium XF	250 ml	C-28070
Cancer Cell Line Medium XF	250 ml	C-28077
Cryo-SFM	30 ml	C-29910
Accutase Solution	100 ml	C-41310
Dulbecco's PBS, without Ca^{++}/Mg^{++}	500 ml	C-40232

**not available as single item*

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