Troubleshooting guide for isolation of patient-derived primary cancer cells

PromoCell®

Possible challenges	Possible reason	Suggested solutions
Low initial cell yield 	Insufficient tumor tissue	Start with minimum 0.2–3 gr of fresh tumor tissue; ≥1 gr is optimal.
	Tissue not homogenized properly	Dissect the tumor tissue into small pieces (approximately 1 mm^3) using a scalpel; do this in the presence of Primary Cancer Culture System (PCCS), and avoid excessive attrition.
	Tissue loss in washing step	Retrieve floating homogenized tissue from the washing buffer using a suitable cell strainer.
	Incorrect centrifugation settings when pelleting single cell suspension	Pellet the single cell suspension for 10 min at 240 x g at room temperature; aspirate only the supernatant without disturbing or discarding the cell pellet.
Contamination during preparation 	Aseptic techniques not followed	Use gentamicin at 50 µg/ml; for antifungal needs, use 10 µg/ml caspofungin. **Note:** Other antibiotics like penicillin or streptomycin inhibit the growth of primary cells, including primary cancer cells.
Residual blood and debris in the sample 	Insufficient washing of the tissue	Wash the homogenized tissue with 10x volume of PBS multiple times until the supernatant is clear.
	Insufficient separation after digestion	After enzymatic digestion, filter turbid supernatant using cell strainers of descending pore size and discard remaining tissue pieces.
Incomplete digestion of tumor tissue 	Insufficient incubation time or mixing	Incubate the tissue in a tissue digestion solution at room temperature with gentle mixing until the solution becomes turbid (typically 30–45 minutes). Do not exceed 60 minutes to avoid compromising cell viability.

Follow these troubleshooting steps to improve the isolation and maintenance of patient-derived primary cancer cells using the Primary Cancer Culture System. For issues not covered here, contact our Cancer Specialists. Refer to the Instruction Manual for detailed step by step protocol. This guide supplements the Application Note and Instruction Manual. It is not a stand-alone file.

Possible challenges	Possible reason	Suggested solutions
Low viability of isolated cells 	Harsh pelleting or improper resuspension	Pellet the cell suspension gently and resuspend in Primary Cancer Culture System (PCCS). Plate the cells at the recommended density.
	The tumor tissue is either not fresh enough or has been damaged by radiation or chemotherapy	Ensure the tumor tissue is as fresh as possible, ideally within 6 hours of surgical removal. Store the tissue in HBSS at 2–8°C immediately after removal. If possible, consider the recent treatment the tissue donor underwent.
	Over-digestion of the tumor tissue	Digest the tissue until the solution becomes distinctly turbid. Do not digest the tissue longer than 60 minutes.
Uneven cell distribution in culture vessels 	Inadequate mixing of cell suspension	Ensure thorough mixing of the cell suspension before plating, and use the appropriate volume of medium per cm^2 of culture surface.
Slow or no cell growth 	Medium exhaustion or contamination	Add fresh medium without antibiotics on days 5–7 and monitor the culture closely. If the medium turns orange-yellow, add fresh medium sooner. In case of contamination, follow the steps listed under "Contamination during preparation" above.
	No viable cancer stem cells	If the tissue was damaged by radiation or chemotherapy, there may not be enough cancer stem cells in your culture. Discard isolations where no viable primary culture is established within six weeks.
	Use of antibiotics	Many antibiotics inhibit the growth of primary cells, including primary cancer cells. If needed, use gentamicin (50 µg/ml) or caspofungin (10 µg/ml).
Loss of cancer stem cell population 	Non-selective culture conditions	The Primary Cancer Culture System (PCCS) selectively supports the growth of cancer stem cells, which ultimately outgrow non-malignant cells. Monitor the culture for the formation of multicellular aggregates and separate them as needed. Work in a serum-free environment.
	Ineffective NCCD treatment	Dilute the thawed NCCD reagent 1:20 with PBS and ensure it covers the entire culture surface for homogenous treatment (100 µl/cm^2). Leave the vessel closed for at least one hour at room temperature. Aspirate the NCCD solution just before seeding the cells.
Difficulty passaging cells 	Improper detachment or sedimentation techniques	For adherent cells, use Accutase for detachment and perform a 1:1 or 1:2 split. For suspension cultures, use gravity sedimentation to reduce debris.

Follow these troubleshooting steps to improve the isolation and maintenance of patient-derived primary cancer cells using the Primary Cancer Culture System. For issues not covered here, contact our Cancer Specialists. Refer to the Instruction Manual for detailed step by step protocol. This guide supplements the Application Note and Instruction Manual. It is not a stand-alone file.

Possible challenges	Possible reason	Suggested solutions
Loss of cell viability during long-term culture	Suboptimal culture conditions	Maintain the culture in Primary Cancer Culture System (PCCS) and use NCCD-treated vessels. Avoid using serum or non-defined supplements. Consider switching to an expansion medium (C-28070 or C-28077).
	Infrequent medium changes	Replace the medium completely every 10–14 days. Use the appropriate technique based on the growth pattern of the primary cells (adherent or suspension).
Contamination with non-cancerous cells	Incomplete depletion of non-malignant cells due to ineffective NCCD treatment	Passage the culture multiple times in Primary Cancer Culture System (PCCS) using NCCD-treated vessels to deplete non-malignant cells. Dilute the NCCD reagent 1:20 with PBS and ensure it covers the entire culture surface. Leave the vessel closed for at least one hour at room temperature.

Follow these troubleshooting steps to improve the isolation and maintenance of patient-derived primary cancer cells using the Primary Cancer Culture System. For issues not covered here, contact our Cancer Specialists. Refer to the Instruction Manual for detailed step by step protocol. This guide supplements the Application Note and Instruction Manual. It is not a stand-alone file.

Contact our experts

Dr. Alexander Trampe is one of our scientific support specialists for the cancer media toolbox. With a Ph.D. in cellular biology and over 14 years of experience in supporting cancer researchers with cell culture and cell analysis applications, he knows how important the development of new cancer therapies is.

Not sure which of our cancer media toolbox options is right for you?
Contact our expert, Dr. Alexander Trampe *(alexander.trampe@promocell.com)*.
Together, you can explore your intended use and other potential applications, and he can provide you with tips and information to help you make the most of the cancer media toolbox.

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

info@promocell.com
www.promocell.com

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 – 90 93 32 (ligne verte)
Téléfax: 0800 – 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 96 03 33 (toll free)
Fax: 0800 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

01/2025