Miltenyi Biotec

TumorMACS™ Media

Frequently asked questions

What are the benefits of TumorMACS Media over home-brew media?

Novel tumor cell lines are often derived from primary tissues in order to use early passages as models in cancer research. However, using home-brew media, this process is inefficient for most tumor entities. Many home-brew media contain large amounts of mostly undefined serum. Serum as a supplement exerts selective pressure leading to a reduction of clonal heterogeneity in primary tumor cell cultures and driving primary tumor cells to a more differentiated state[1–3].

Alternatively, established tumor cell lines are available as tumor model systems. However, many tumor subtypes are not represented by established tumor cell lines since home-brew media fail to support the growth of certain tumor cells. There is, for example, no cell line available for the exocrine-like subtype of pancreatic adenocarcinomas so far.

To address these challenges, Miltenyi Biotec developed specialized serum-free media, our TumorMACS Media, which enable the initiation and expansion of primary tumor cell cultures from epithelial tumors over multiple passages[4]. TumorMACS Media are designed to support the unbiased growth of epithelial tumor cells from primary and xenotransplanted tissue by enhancing the cells' ability to proliferate without inducing any genomic, transcriptomic, and/or metabolomic changes. Thus, TumorMACS Media enable a gentle selection of cancerous cells while preserving the initial heterogeneity of the bulk tumor.

TumorMACS Media have been optimized with regard to formulation, stability, and ease of use. They support the culture of cells of a specific tumor entity, independent of the respective tumor subtype. Pancreas TumorMACS Medium, for example, allows the generation of cell lines from classical, quasimesenchymal, and exocrine-like pancreatic ductal adenocarcinoma (PDA) as defined according to the PDAssigner[5] genes.[4]

Furthermore, high lot-to-lot consistency of TumorMACS Media guarantees reproducible conditions for reliable analysis. It also saves precious hands-on time otherwise required to design, standardize, and validate the proper formulation of home-brew media for the nutritional requirements of tumor cells of different tumor entities.

Notably, to streamline tumor cell culture, TumorMACS Media offer the flexibility to seed tumor pieces, single cells from bulk tumor, or pre-sorted populations of tumor cells.

What do I have to pay attention to when generating and expanding primary tumor cell cultures with TumorMACS Media?

TumorMACS media have been designed for improved and convenient primary tumor cell culture. Still, there are multiple factors that influence the final success rate. Of great importance are proper sample handling, type of sample, processing speed, cell density, and plating procedure, as well as culture surfaces and coating, presence or absence of fibroblasts, presence or absence of serum, freeze-thaw procedures, and media exchange. In order to fully support you during these crucial steps, we have developed a standardized workflow covering tissue dissociation, seeding of purified tumor cells, and tumor cell propagation.

VISIT

A detailed guideline is available at

▸ **miltenyibiotec.com/tumorculture**

Do TumorMACS Media support primary cell expansion in both adherent and suspension tumor cell cultures?

Yes, TumorMACS Media support the growth of both adherent and suspension cell cultures. Spheroid-like structures may spontaneously emerge if the intrinsic characteristics of the individual tumor promote the formation of these structures (fig. 1). This is of particular importance as even epithelial tumor cell samples might grow better in a suspension culture of spheroid-like structures than in an adherent culture (fig. 1B vs. fig. 1A). Moreover, some cell lines may shift between an adherent and a suspension state upon passaging. Accordingly, cultures need to be carefully inspected, especially after passaging, and floating cells should not be discarded right away.

VISIT

For further details on the formation of suspension culture and best cultureware surfaces and coating, please refer to the "Guide for the initiation of primary tumor cell cultures" available at

▸ **miltenyibiotec.com/tumorculture**

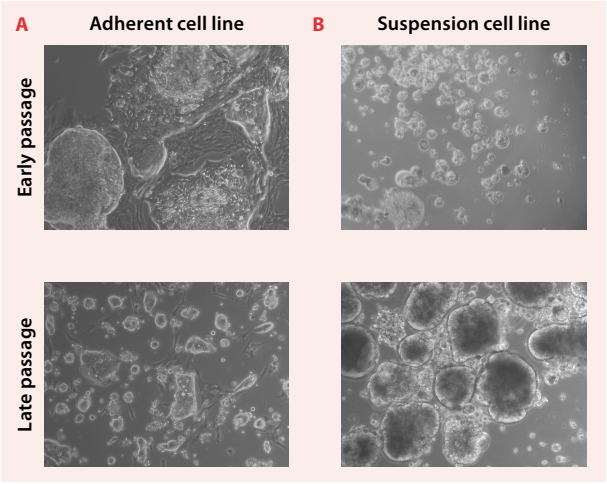

Figure 1: Establishment of adherent or suspension culture.
The images show two different cell lines, derived from the same primary pancreatic adenocarcinoma, growing in adherent or suspension culture. During initial cultivation of the primary tumor cells, some cells detached, grew in suspension (B), and gave rise to a separate cell line, distinct from the cells that kept their adherent characteristics (A). Pancreas TumorMACS Medium supported cell expansion both in adherent and suspension cultures. Remarkably, in the samples shown, the epithelial tumor cells grew better in a suspension culture of spheroid-like structures (B vs. A).

What is the best way to adjust my already existing primary tumor cell culture to TumorMACS Media?

Be patient. Exchanging the culture medium can be stressful for your cells and they might require some time to adapt to the new environment, particularly if the previous medium contained serum. We therefore recommend a gradual transition to TumorMACS Medium by starting with 75% previous medium plus 25% TumorMACS Medium on proliferating cells (cells that started to expand after passaging). If the cells respond to this change with a normal growth, increase the amount of TumorMACS Medium gradually during the proliferation phase after each passaging step to 50%, 75%, and finally to 100% TumorMACS Medium.
In case you notice stress symptoms in your cells, we recommend a slower transition starting with 10–15% TumorMACS Medium diluted in previous medium. Continue with a gradual transition until only TumorMACS Medium is used.

How efficiently can TumorMACS Media support the generation of primary tumor cell cultures?

A number of factors significantly affect the success rate when generating a primary tumor cell culture. Please refer to paragraph "What do I have to pay attention to when generating and expanding primary cell cultures with TumorMACS Media?" for further details. TumorMACS Media can effectively support you in this challenging task enabling a success rate of up to 93% for generating and expanding primary tumor cell cultures over a period of 2 weeks (table 1).

Tumor entity	Success rate*
Pancreatic	86% (19/22)
Ovarian	93% (14/15)
Renal	85% (6/7)
Colon	90% (28/31)

Table 1: Success rate for generating primary tumor cell cultures using TumorMACS Media.
* Data were generated on specimens shipped and stored overnight. Maintained up to 2 weeks.

To learn how TumorMACS Media can further help you to generate stable primary cell lines from primary tumor cell cultures, please refer to paragraph "How efficiently can TumorMACS Media support the generation of stable primary tumor cell lines starting from primary cultures?".

How efficiently can TumorMACS Media support the generation of stable primary tumor cell lines starting from primary tumor cell cultures?

A spontaneous establishment of a cell line from a primary cell culture can be quite challenging due to demanding environmental conditions. Moreover, some primary tumor cell cultures can simply not be used to generate cell lines due to several (epi)genetic factors. Please read paragraph "What do I have to pay attention to when generating and expanding primary cell cultures with TumorMACS Media?" for further details. To date, the success rate with home-brew media described by the literature ranges between less than 9% and a maximum of 46%, also depending on the tumor entity. The serum-free formulation of TumorMACS Media is superior to home-brew media for the generation of primary cell lines (table 2) by enhancing cell proliferation without inducing any genomic, transcriptomic, or metabolomic changes.

Tumor entity	Success rate		
	TumorMACS Media*	Home-brew media	References**
Pancreatic	50% (12/24)	9%	6–9
Ovarian	32% (6/19)	12%	10
Renal	50% (3/6)	22%	11
Colon	74% (17/23)	10%	6, 12–14

Table 2: Success rate for generating primary tumor cell lines with tumor-specific TumorMACS Media versus home-brew media.
*Data were generated on specimens shipped and stored overnight. Maintained for >10 passages, freeze-thaw cycle possible.
**References refer to success rate for generating primary tumor cell lines with home-brew media.

References

1. Ricci-Vitiani L. *et al.* (2007) Identification and expansion of human colon cancer–initiating cells. Nature 445(7123): 111–115.
2. Todaro M. *et al.* (2007) Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. Cell Stem Cell 1(4): 389–402.
3. Lee J. *et al.* (2006) Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer Cell 9(5): 391–403.
4. Noll E.M. *et al.* (2016) CYP3A5 mediates basal and acquired therapy resistance in different subtypes of pancreatic ductal adenocarcinoma. Nat. Med. 22(3): 278–287.
5. Collisson E.A. *et al.* (2011) Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat. Med. 17(4): 500–503.
6. Byrne A.T. *et al.* (2017) Interrogating open issues in cancer medicine with patient-derived xenografts. Nat. Rev. Cancer 17(10): 632. Epub ahead of print, Sep. 15.
7. Rückert F. *et al.* (2012) Molecular mechanism of the "feedback loop" model of carcinogenesis. Commun. Integr. Biol. 5(5): 506–507.
8. Boj S.F. *et al.* (2015) Model organoids provide new research opportunities for ductal pancreatic cancer. Mol. Cell Oncol. 3(1): e1014757.
9. Huang T. *et al.* (2015) The emerging role of Slit-Robo pathway in gastric and other gastro intestinal cancers. BMC Cancer 15: 950.
10. Verschraegen C.F. *et al.* (2003) Establishment and characterization of cancer cell cultures and xenografts derived from primary or metastatic Mullerian cancers. Clin. Cancer Res. 9(2): 845–852.
11. Yap N.Y. *et al.* (2019) Establishment of epithelial and fibroblast cell cultures and cell lines from primary renal cancer nephrectomies. Cell Biol. Int. 43(6): 715–725.
12. Dangles-Marie V. *et al.* (2007) Establishment of human colon cancer cell lines from fresh tumors versus xenografts: comparison of success rate and cell line features. Cancer Res. 67(1): 398–407.
13. Weeber F. *et al.* (2015) Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. Proc. Natl. Acad. Sci. U.S.A. 112(43): 13308–13311.
14. van de Wetering M. *et al.* Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 161(4): 933–945.

Miltenyi Biotec

Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macsde@miltenyi.com | **www.miltenyibiotec.com**

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

V.01