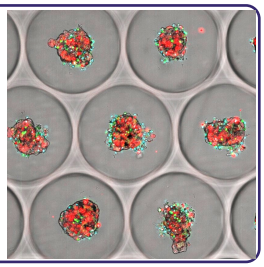
Application note

T Cell Killing Assay on Gri3D®

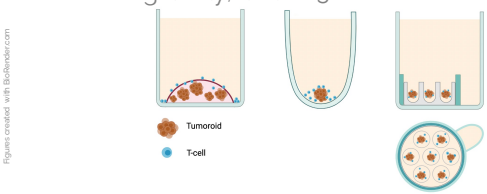
Introducing T cell killing on Gri3D®

Gri3D® is a ready-to-use platform for high-throughput and reproducible organoid culture. In collaboration with scientists at the École Polytechnique Fédérale de Lausanne, we set up a robust and scalable assay to study tumor-infiltrating lymphocytes (TIL) functionality on 3-dimensional (3D) tumoroids grown in Gri3D®. Up to 73 tumoroids are formed in a single well and subsequently co-cultured with TILs. Evaluation of T cell killing potential is performed *in situ* via semi-automated image analysis.



T cell killing assays: state of the art

With the recent developments in 3D cell culture and organoid technology, multiple T cell killing assays have been established to test T cell functionality and response to immunotherapies *in vitro*. However, current assay systems are limited in throughput and difficult to handle. Establishing a controlled co-culture is cumbersome, resulting in low reproducibility of these systems. Therefore, there is a need for a more robust and scalable T cell killing assay, and Gri3D® meets the requirements.

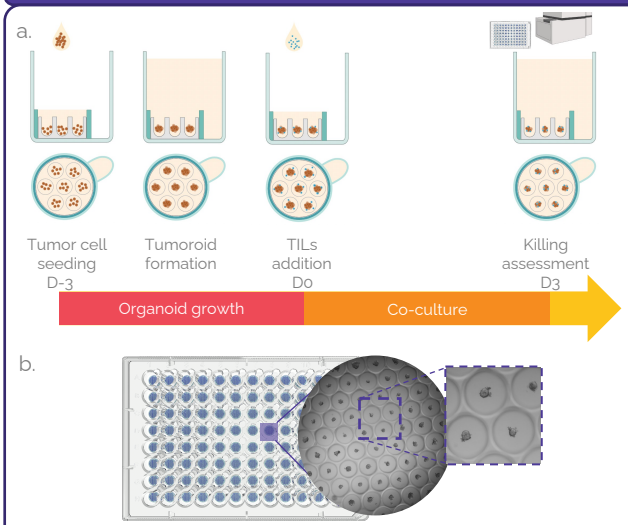


Schematic representation of in vitro T-cell killing assay systems. From left to right: ECM-embedding (e.g. Matrigel), non-adherent surface and Gri3D®.

	ECM-embedding	Non-adherent surface	Gri3D®
Organoid size homogeneity	■	■■	■■■
Focal plane	■	■■■	■■■
Retrievability	■	■■■	■■■
Cell ratio control	■	■■■	■■■
Cell-cell contact	■	■■	■■■
Throughput	■	■■	■■■

Comparative table of in vitro image-based T cell killing assays and Gri3D®.

T cell killing assay on Gri3D®



a. *Workflow for the generation of tumoroids and assaying of TILs on Gri3D®.*
b. *Representative brightfield image of human colorectal tumoroids grown on Gri3D® 500 µm microwells .*

T cell killing assay procedure:

1. Gri3D® 96WP 500 µm microwells are used to generate **tumoroids of a defined size**.
2. Once the organoids have formed, **TILs are added** at various effector to target (E:T) ratios and in presence or absence of immunomodulators.
3. The evolution over time of both cell types is followed with cell trackers.
4. On the **assay** day, **propidium iodide** is added to assess killing
5. Finally, **semi automated image analysis** is used to extract valuable parameters such as cell death, tumor size or TIL migration.

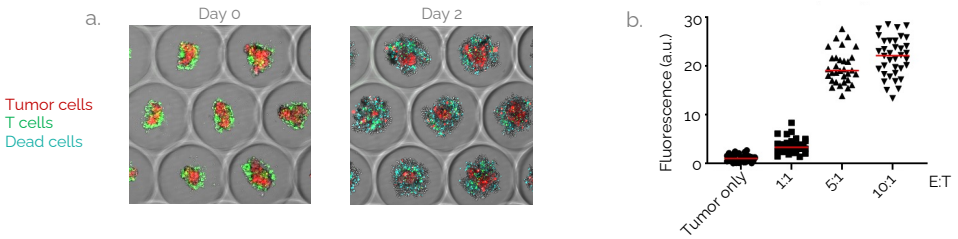
Order yours
www.SUNBIOSCIENCE.ch

Contact us
enquiries@sunbioscience.ch

Address
EPFL Innovation Park
Bâtiment D
CH-1015 Lausanne

T cell killing assay validation

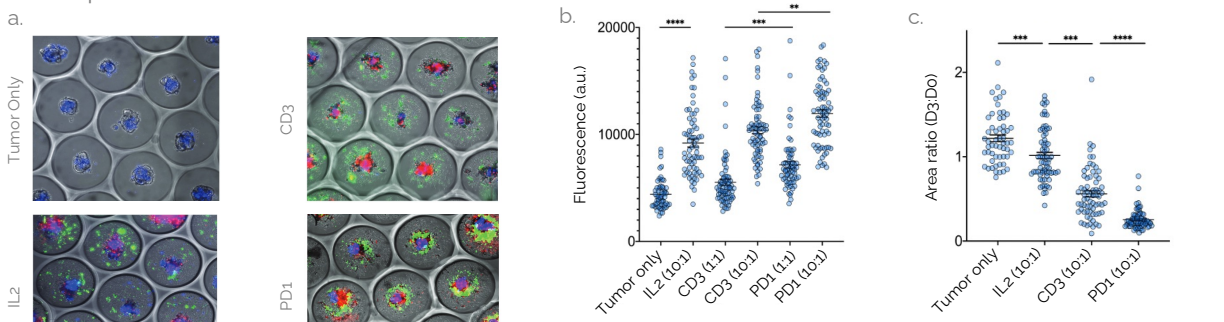
The assay is validated using the Pmel-1 transgenic mouse model. Melanoma tumoroids grow steadily in absence of T-cells but are disrupted in the presence of T cells. Higher effector to target ratios (E:T) allow more efficient tumor killing, as observed by increasing propidium iodide signal.



T cell killing assay on pMEL-1 mouse model ***a.*** *Respresentative images of the co-culture of B16-F10 melanoma tumoroids with targeting T cells at 10:1 E:T. Scale bar: 500 µm.* ***b.*** *Propidium iodide quantification at day 2 for different E:T.*

Autologous T cell killing assay

The assay is used to evaluate the killing capacity of patient-derived TILs to autologous human colorectal cancer tumoroids. Moreover, TIL addition is combined with immunomodulators. This selected patient sample shows autonomous T cell activation, and the cytotoxic response is increased in presence of immune checkpoint inhibitors.



Co-culture of patient-derived colorectal tumoroids and autologous TILs and assessment of tumor death and shrinkage after treatment with immunomodulators. ***a.*** *Images of the assay at day 3 showing tumor cells (blue), T cells at 10:1 E:T ratio (green) and dead cells (red). Scale bar: 500 µm.* ***b.*** *Quantification of PI staining intensity at day 3.* ***c.*** *Quantification of tumoroid area at day 3.* ***CD3****: IL-2 + α-CD3/CD28.* ***PD1****: IL-2 + α-CD3/CD28 + α-PD1/CTLA4.*

Highlights of the model

- **Robust**: Gri3D® establishes controlled co-cultures of tumoroids in direct contact with T cells.
- **Maximized contact** between T-cells and tumoroids in a close to *in vivo* manner.
- **Scalable**: using the Gri3D® T cell killing assay, up to 73 homogeneous tumoroids are evaluated in a single well, significantly increasing the number of datapoints per well.
- **Relevant**: the use of patient-derived tumoroids and autologous TILs enables patient-specific assays.

How can we help you?

T-Cell Killing Assay Service
SUN bioscience generates organoids where the killing potential of T cells can be assessed in high throughput.
Contact us for more information at enquiries@sunbioscience.ch

References

The data reported were generated by Devanjali Dutta, François Rivest and colleagues at École Polytechnique Fédérale de Lausanne. Find out more in the pre-print: Dutta, D. et al. Probing the killing potency of tumor-infiltrating lymphocytes on microarrayed autologous tumoroids. bioRxiv 1–19 (2021) doi.org/10.1101/2021.03.30.437679.

SUNBIOSCIENCE

Order yours
www.SUNBIOSCIENCE.ch

Contact us
enquiries@sunbioscience.ch

Address
EPFL Innovation Park
Bâtiment D
CH-1015 Lausanne