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How does SpheroTribe work?

- SpheroTribe relies on the use of methylcellulose, a crowding agent that is well-known to drive efficient cell aggregation into spheroids. Once diluted in culture medium, methylcellulose increases the viscosity of the medium, creating a gel-like environment that restrict cell movement and encourages cell-cell contacts for the formation of compact spheroids.

Can SpheroTribe affect my cell physiology or subsequent assays?

- The SpheroTribe solution is composed of methylcellulose, a derivative of cellulose that is known to be biologically inert, i.e. to neither actively contribute to biological responses nor interfere with it. It is notably used in various biofabrication tools, including bioinks. In addition, SpheroTribe allows you to culture cells without exogenous ECM components, avoiding the known bias they may have on cell signaling. If needed, SpheroTribe can be readily washed off at any stage of the protocol to leave spheroids available for subsequent assays.

Which cells has SpheroTribe been used with?

- So far, SpheroTribe has been successfully used to generate spheroids/organoids with the following cell types:
    - Patient-derived stem-like glioblastoma cells (GB P3, BL13, T033 and T042),
    - human glioblastoma cell lines (U87 and T98G),
    - HeLa,
    - human vaginal mucosal melanoma (HMV-II),
    - human primary colorectal cancer cells,
    - human breast cancer cells (MDA-MB 231),
    - monkey kidney fibroblast-like cell line (COS-7),
    - primary neurons from rat embryos (E18)
    - murine melanoma cells (B16F10),
    - human induced pluripotent stem cells (hiPSCs)-derived organoids.

Is there a particular medium I need to use SpheroTribe with?

- The SpheroTribe concentrated solution is made up in basal medium and does not contain any proteins, lipids or growth factors. You can dilute it in any culture medium of your choice, and add other compounds as desired (i.e. serum, antibiotics, etc.).

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How many experiments can I perform with one SpheroTribe kit?

- One SpheroTribe kit contains everything you need to grow up to 960 unique spheroids. Total number of spheroids generated with one kit may vary depending on the concentration of SpheroTribe solution used for cell aggregation, total spheroid growth duration and frequency of medium renewal.

Protocol of Use

How long will it take to grow spheroids with SpheroTribe?

- The total time to initiate 3D cell culture with SpheroTribe should take no more than 30 minutes. After that, optimal culture duration to achieve desired spheroid size will vary depending on your cell proliferation rate and targeted application. As a general rule, compact and homogeneous spheroids are usually formed after 3 days of culture.

How many cells should I seed in each well?

- Optimal seeding density can vary greatly depending on the cell type, proliferation rate and targeted application. You will most probably need to start with a couple of optimization tests to find out which density will best suit your needs. As a general rule, we recommend using 10,000 to 20,000 cells per well for primary cells and 2,000 to 7,000 cells per well for tumoral/immortalized cell lines.

How big will my spheroids get?

- Spheroid sizes can vary greatly depending on cell type characteristics, initial density and culture conditions used. To help you select optimal conditions, here are a couple of examples of sizes reached for different cell types and seeding densities:

Cell type	Seeding density (nb of cells/well)	Day 2	Day 3	Day 4	Day 6
Patient-derived glioblastoma	10,000			450-500µm	
HMV-II	10,000	700µm	1,500µm		
	7,000			2,300µm	
T98G	7,000			700µm	
B16F10	10,000				1,200µm

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Do you have specific recommendations for downstream assays?

- A variety of downstream assays have been performed with spheroids generated with SpheroTribe. The manufacturer has only provided a detailed protocol for the formation of spheroids, as this is what SpheroTribe is designed for. However, if needed, you can check out the following publications describing protocols for various assays using glioblastoma spheroids generated with SpheroTribe. These can easily be applied to other cell types:

    - Invasion assay + Fiji macro for easy quantification of invasion, migration and proliferation: Guyon, J., Andrique, L., Pujol, N., Røsland, G. V., Recher, G., Bikfalvi, A., Daubon, T. A 3D Spheroid Model for Glioblastoma. J. Vis. Exp. (158), e60998, doi:10.3791/60998 (2020).
    - Tumor spheroid immunostaining and mice intracranial implantation: Guyon, J. et al, Lactate dehydrogenases promote glioblastoma growth and invasion via a metabolic symbiosis. EMBO Mol Med (2022) 14/ e15343, doi:10.15252/emmm.202115343.

How can I make sure my spheroids will be unique and uniform?

- The SpheroTribe kit has been shown to generate unique and uniform spheroids with most cell types tested. Yet, when working with particularly challenging cells, here are a few tips to keep in mind to maximize your chance to obtain unique and uniform spheroids in each well:

    - Starting from a single-cell suspension: the presence of cell clumps in your initial cell suspension at the time of seeding can compromise the obtention of unique and uniform spheroids. When starting from isolated primary cells, we recommend that you carefully dissociate cells using filters or columns if needed.
    - Pre-heating the SpheroTribe solution at 37°C before use: lower temperatures will make it more viscous, potentially favoring the formation of multiple cell aggregates.
    - If the above advice is not sufficient, centrifuging the plate at 300g for 3 minutes can help with spheroid formation.

Any other questions? Do not hesitate to contact us.