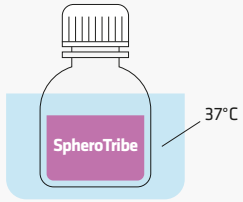
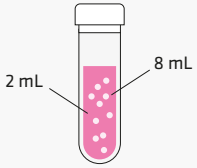
How to use SpheroTribe in pictures

A



Pre-heat the SpheroTribe solution at 37°C in a water-bath.

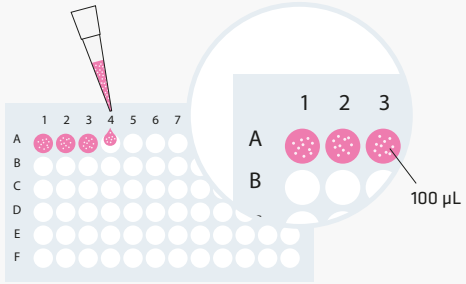
B



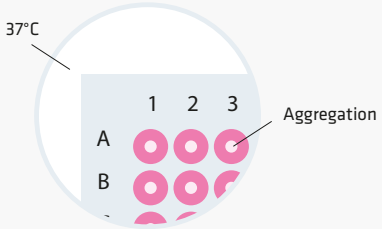
To get 100 spheroids of 10^4 cells each, mix 2 mL of SpheroTribe + 8 mL of cell suspension containing 10^6 cells.

C

Dispense 100 µL of the cell suspension in SpheroTribe per well.

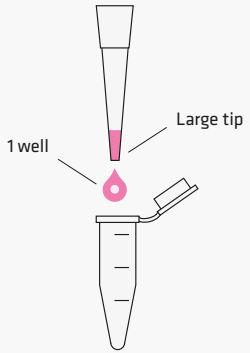


D



Incubate for 3 – 4 days at 37°C, 5% CO_2 for cell aggregation.

E



Keep in the plate or collect for downstream analysis with the large-opening pipette tip.

Check videos of protocol, examples of results and much more on:
idylle-labs.com/spherotribe

A protocol designed in April 2023

SpheroTribe

PROTOCOL

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idylle

1. The material you need

REAGENTS

- SpheroTribe methylcellulose solution 2% in DMEM (included in the kit)
- DPBS 1X
- Cells & culture medium of your choice

CONSUMABLES

- 96-well round bottom plates (included in the kit)
- Large-opening pipette tips (included in the kit)
- Regular 200 µL pipette tips

2. Storing SpheroTribe

- The SpheroTribe methylcellulose solution 2% in DMEM stock solution is stable for up to 6 months when stored at 4°C.

3. Making spheroids with SpheroTribe

This protocol allows the preparation of 100 spheroids with a final concentration of 0,4% methycellulose. This concentration has been shown to produce uniform and compact spheroids in various cell types (stem-like glioblastoma, COS-7 and HeLa cells). However, the percentage of methylcellulose can be tuned according to the cell type, experimental setup and desired spheroid properties. The number of cells per spheroid can be adjusted according to the preferred size.

- Pre-heat the SpheroTribe solution at 37°C in a water-bath (A).
- To prepare 100 spheroids of 10^4 cells each, prepare 8 mL of a cell suspension in the culture medium of your choice at 125 000 cells/mL.

SpheroTribe can be added to any kind of culture medium and can be used in association with supplements, serum and antibiotics.

Make sure your cell suspension is free of clumps. If working with tumor cells or cells that spontaneously form spheroids in culture, use a dissociation enzyme and incubate for 5 minutes at 37°C before resuspending in your final culture medium to make sure cells are well-separated.

- Add 2 mL of SpheroTribe to the 8 mL of cell suspension to get a final concentration of 100 000 cells/mL (B).
- Homogenize well by pipetting up and down.
- In a 96-well round bottom plate included in the kit, dispense in each well 100 µL of the cell suspension in SpheroTribe (C).

Keep homogenizing your cell suspension regularly while loading the wells to make sure that an equal number of cells is added to each well. Alternatively, a multichannel pipette can be used to fill the wells.

- Incubate the plate at 37°C, 5% CO_2 (D).
- Grow the spheroids for 3-4 days.
- If you grow them for a longer period of time, renew culture medium every 3-4 days (see the renewing medium for long-term spheroid culture section*).

4. Collecting spheroids for downstream applications

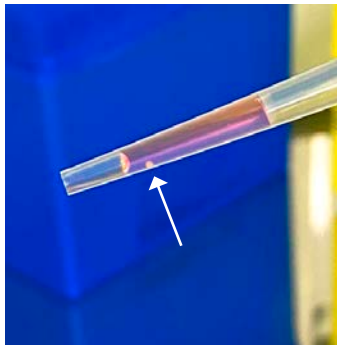
Always use the large opening pipette tips provided when handling the spheroids. Aspirating or releasing them with a regular pipette tip will lead to spheroid disintegration.

- Place the tip at the center of the well and slowly aspirate to collect the spheroid.

Avoid direct contact between the tip and the spheroid to preserve its structural integrity.

Placing your plate over a dark background will help you locate the spheroids in the wells.

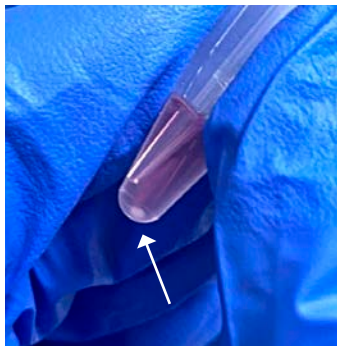
- Make sure the spheroid has been successfully collected by spotting the spheroid inside the tip.



- Slowly release the culture medium containing the spheroid into an Eppendorf tube (E).
- Wait for the spheroid to settle at the bottom of the tube.

If some air is trapped at the bottom of the tube, gently tap to bring the liquid down and allow the spheroid to settle.

- Aspirate slowly using a regular pipette tip to remove the medium without disturbing the spheroid.



Make sure you don't disturb the spheroid by leaving a few microliters of liquid at the bottom of the tube.

- Add 300 µL of DPBS to wash the spheroid. Wait for the spheroid to settle at the bottom of the tube.
- Aspirate slowly using a regular pipette tip to remove the DPBS without disturbing the spheroid.
- Add your culture medium or solution of choice.
- Spheroids can be used directly in the tube or transferred to your culture vessels of choice according to downstream applications.

To transfer spheroids into a flat-bottom plate, place your pipette tip at the center of the well. Slightly lift your tip so that it does not directly come into contact with the plate bottom, and slowly release the spheroid inside the well. Do not press completely to avoid the formation of bubbles.

***Renewing medium for long-term spheroid culture**

- Prepare fresh SpheroTribe culture medium by diluting some pre-heated SpheroTribe solution in your culture medium of choice at a similar ratio as used for spheroid formation (e.g. 2 mL of SpheroTribe for 8 mL of cell suspension).
- Tilt the plate slightly, locate your spheroid inside the well and place your pipette tip on the opposite side. Aspirate slowly to remove the medium without disturbing the spheroid.

Placing your plate over a dark background will help you locate the spheroids in the wells.

- Wash your spheroids by gently adding 200 µL DPBS using an individual or a multichannel pipette. Wait for the spheroids to settle at the bottom of the wells, and aspirate slowly to remove the DPBS without disturbing the spheroid.
- Slowly load 100 µL of fresh culture medium added with SpheroTribe solution per well and place the plate back in the incubator.