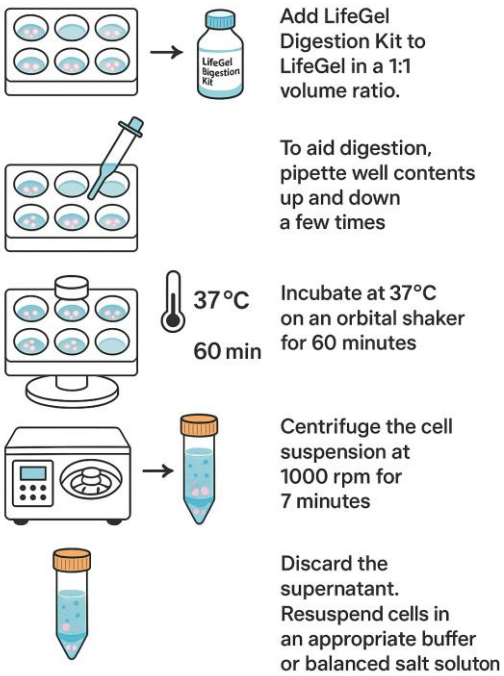
Cells/Spheroids harvesting

PROTOCOL

LifeGel® Digestion Kit
Tissue Digestion Kit

LifeGel Digestion Kit [RRLDK4] enables quick and gentle release of single cells cultured on LifeGel, while preserving the highest possible yield. The kit (4 × 4 mL) is ready to use and sufficient for the digestion of a 48-well, 96-well, or 384-well plate.

Tissue Digestion Kit [RRTDK4] enables quick and gentle release of single cells from primary tissue, while preserving the highest possible yield. The kit (4 × 4 mL) is ready to use and sufficient for the digestion of up to 16 g of tissue.



Add LifeGel Digestion Kit to LifeGel in a 1:1 volume ratio.

To aid digestion, pipette well contents up and down a few times

Incubate at 37°C on an orbital shaker for 60 minutes

Centrifuge the cell suspension at 1000 rpm for 7 minutes

Discard the supernatant. Resuspend cells in an appropriate buffer or balanced salt soluton

Features:

- Gentle and efficient singel cel harvesting – allow to collect single cel suspention from 3D spheroid or tissue fragment.
- Easy-to-use.
- Stable formulation – stable for 6 months in -20°C or until expiration date in -80°C.

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PROTOCOL

LifeGel Digestion Kit

RRLDK4

Content : 4 vials x 4 mL of digestion solution (containing Collagenase types I and IV, and Hyaluronidase). Product sufficient for digestion of a 48-well plate.

Storage: Store at -20°C for 6-month stability or at -80°C for stability until expiry date. Protect from light. Avoid repeated freezing and thawing.

Recommended materials and reagents:

- 15 ml centifuge tubes
- 48-well or 96-well plate
- Serological pipettes
- Orbital shaker
- Centrifuge

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1. Remove the medium covering the LifeGel surface.*
2. Add LifeGel Digestion Kit to LifeGel in a 1:1 volume ratio. (For 48-well 300 µL, for 96-well 150 µl).
3. To aid digestion, pipette well contents up and down a few times.
4. If most or all of the plate is to be digested, incubate it at 37°C on an orbital shaker (e.g. 30-60 rotations per minute) for 60 minutes. If only a few wells are to be digested, transfer well contents to a vial and vortex every 15 minutes for 60 minutes (time depends on cell line and size of spheroids).
5. Centrifuge the cell suspension at 1000 rpm for 7 minutes. Discard the supernatant. Resuspend cells in an appropriate buffer or balanced salt solution.
6. Repeat the centrifugation of the cell suspension and resuspend cells in an appropriate cell culture medium.

* Be careful when culture medium to avoid disturbing the gel.

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Tissue Digestion Kit

RRTDK4

Content : 4 vials x 4 mL of digestion solution (containing Collagenase types I and IV, and Hyaluronidase). Product sufficient for digestion of a 48-well plate.

Storage: Store at -20°C for 6-month stability or at -80°C for stability until expiry date. Protect from light. Avoid repeated freezing and thawing.

Recommended materials and reagents:

- Scalpel or scissors
- 15 ml and 50 ml centifuge tubes
- 48-well plate
- Cell strainer (70 µm)
- Orbital shaker
- Centrifuge

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1. Using a sterile scalpel or scissors, cut tissue into 1-4 mm pieces. Weigh them separately.
2. Wash the tissue with an appropriate buffer or a balanced salt solution (e.g. PBS).
3. Transfer the tissue fragments into a 48-well plate or suitable tubes containing the appropriate amount of the Tissue Digestion Kit (1 mL per 1 g of tissue).
4. Incubate at 37°C on an orbital shaker (e.g. 30-60 rotations per minute) for 60 minutes or vortex every 10 minutes.
5. Pass the cell suspension through a cell strainer (not included).
6. For dense tissue material (e.g. hard tumor tissue), collect the cells from step 5 in a separate container. The remaining non-digested tissue from the cell strainer can be processed in step 4 for up to 3 times (add 1 mL of the Tissue Digestion Kit per 1 g of tissue or less). Otherwise, skip this step and go to step 7.
7. Centrifuge the cell suspension at 1000 rpm for 7 minutes. Discard the supernatant. Resuspend cells in an appropriate buffer or balanced salt solution or medium.
8. If required, count and seed cells for culture.