

Retrieval of cells from BIOMIMESYS® hydro scaffolds

BIOMIMESYS® technology is a Hyaluronic Acid-based hydro scaffold that allows physiological 3D cell culture. Cells can be easily retrieved with a quick digestion of the hydro scaffold. For a more efficient digestion, the enzymatic digestion is coupled to a mechanical action by continuous gentle shaking. This process does not impact the viability of the cells.

BIOMIMESYS® Cell Retrieval materials:

- Hyaluronidase enzyme from bovine testes
- Detachin™ (sterile cell detachment solution)
- Chlorhydric acid (HCl) 0.1N.

1. Hydro scaffold digestion

Depending of the hyaluronidase powder concentration, adjust the medium volume to obtain a hyaluronidase concentration of **7000IU/ml** and use 750µl per hydro scaffold.

1.1 Weigh the hyaluronidase powder

1.2 Adjust the pH of the culture medium:

- Use pre-warmed culture medium (at room temperature, for at least 30 minutes)
- Without serum and antibiotics
- With HCl 0.1N to pH=5.

Note: phenol red should turn yellow.

1.3 Filter through a 0.2µm or 0.45µm filter to sterilize.

1.4 Solubilize the hyaluronidase in 3ml of culture medium (for 4 hydro scaffolds) in a 50ml tube (see in “further information” for other compatible tubes).

1.5 Vortex the tube for a few seconds to solubilize the enzyme and warm the solution at 37°C for 10 minutes.

1.6 Using fine forceps, place 4 hydro scaffolds in the hyaluronidase solution.

1.7 Place the solution containing hydro scaffolds on an orbital shaker (shaking between 100 and 200 rpm, depending on the magnitude of rotation) at 37°C for 30 to 45 minutes.

Note: Invert the tubes 2 times every 10 minutes to facilitate mixing of the hydro scaffold/enzymatic solution and thereby the digestion of the hydro scaffold.

1.8 Collect the whole suspension and transfer into a new 15ml tube.

1.9 Centrifuge at 190g for 5 minutes at room temperature.

Note: Stop here to recover spheroids or multicellular aggregates if cell dissociation is not needed for further analysis.

2. Spheroid dissociation

2.1 Pre-warm the Detachin™ solution for 10 minutes at 37°C.

2.2 Remove the supernatant (step 1.9) and re-suspend the cell pellet in 2ml of pre-warmed Detachin™. Pipette up and down several times to homogenize the Detachin™ spheroid suspension.

2.3 Incubate for 10 minutes at 37°C.

2.4 Pipette up and down 10 times for a complete dissociation of the spheroids.

2.5 Add 4ml of complete culture medium (containing 10% FCS) to stop the enzyme activity.

2.6 Centrifuge at 190g for 5 minutes at room temperature.

2.7 Remove the supernatant and re-suspend the cells in complete culture medium or PBS buffer depending on the type of experiment planned.

2.8 Filter through a 30µm filter to remove the remaining hydrosccaffold fibers.

Further information:

- ✓ It is important to keep the concentration of hyaluronidase at **7000 IU/mL** to correctly digest the hydrosccaffolds.
- ✓ The enzymatic digestion can be performed in:
 - 50ml or 15ml tube: placed vertically
 - Glass pillbox: placed vertically
 - Eppendorf tube: placed lying down and containing 750µl of hyaluronidase solution and 1 hydrosccaffold
 - Polypropylene tube: placed vertically
- ✓ **We do not recommend the use of culture plates.**
- ✓ **Avoid the use of magnetic stirrer, it would induce cell death.**

Contact Information

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