Retrieval of cells from BIOMIMESYS® hydroscaffolds

BIOMIMESYS® technology is a Hyaluronic Acid-based hydroscaffold that allows physiological 3D cell culture. Cells can be easily retrieved with a quick digestion of the hydroscaffold. 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. Hydroscaffold digestion

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

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 hydroscaffolds) 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 hydroscaffolds in the hyaluronidase solution.

1.7 Place the solution containing hydroscaffolds 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 hydroscaffold/enzymatic solution and thereby the digestion of the hydroscaffold.

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 hydroscaffold fibers.

Further information:

- ✓ It is important to keep the concentration of hyaluronidase at **7000 IU/mL** to correctly digest the hydroscaffolds.
- ✓ 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 hydroscaffold

  - 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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