# Retrieval of cells from BIOMIMESYS® scaffolds

BIOMIMESYS® is a hyaluronic acid-based scaffold that allows physiological 3D cell culture. Cells can be easily retrieved with a quick digestion of the 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 Kit*** (Catalog number: *KIT-HASE-HAx24*) is **designed to digest up to 24 BIOMIMESYS® scaffolds**. It is composed of:

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

## 1. Hydrogel digestion

Depending of the hyaluronidase powder concentration, adjust the medium volume to obtain a hyaluronidase concentration of 5000IU/ml and use 750µl per hydrogel.

1.1 Weigh the hyaluronidase powder (provided with our **BIOMIMESYS®*Cell Retrieval Kit***)

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 pH5 (provided with our **BIOMIMESYS®*Cell Retrieval Kit***)

**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 hydrogels) 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 hydrogels in the hyaluronidase solution.

1.7 Place the solution containing hydrogels 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 hydrogel/enzymatic solution and thereby the digestion of the hydrogel.

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 (provided with our **BIOMIMESYS®*Cell Retrieval Kit***) 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.

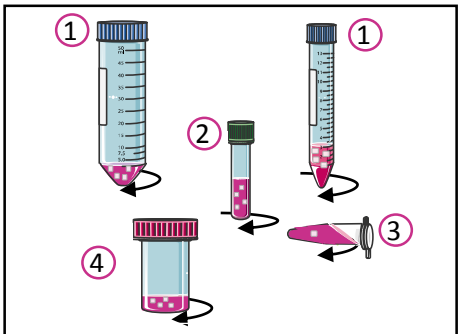
2.6 Centrifuge at 190g for 5 minutes at room temperature.

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 gel fibers.

**Further information:**

- It is important to keep the concentration of hyaluronidase at **5000 IU/mL** to correctly digest the hydrogels.
- The enzymatic digestion can be performed in (see figure on side):
  - 50ml or 15ml tube: placed vertically (1)
  - Glass pillbox: placed vertically (2)
  - Eppendorf tube: placed lying down and containing **750µl of hyaluronidase solution and 1 hydrogel** (3)
  - Polypropylene tube: placed vertically (4)



Tubes types to perform enzymatic digestion

- We do not recommend the use of culture plates.
- Avoid the use of magnetic stirrer, it would induce cell death.
- To digest BIOMIMESYS®*Hepatocyte* and BIOMIMESYS®*Adipocyte* scaffolds with **BIOMIMESYS®*Cell Retrieval Kit***, this protocol should be adjust. Contact us for further information: contact@celenys.com.