



HISTOGEL protocol

This protocol is designed for processing organoids grown in chamber slides (4 or 8 well). The processed histogel blocks will be used for paraffin embedding and generation of sections that contact organoid slices.

Materials:

1. Histogel: Thermo Scientific. Cat. # HG-4000-012
2. 0.5% Eosin in 70% Ethanol
3. Cryomolds : Tissue-tek cryomold standard 4557 (10x10x5mm)
4. 10 % Neutral Buffered Formalin. Sigma Aldrich HT501128
5. 4% Paraformaldehyde (see below for a recipe)

Procedure:

Prior to starting the Histogel protocol, warm the Histogel in 65°C water bath for 1 to 2 hours until it liquefies. It is important to keep Histogel at 65°C, a drop in temperature by more than 3° is sufficient to harden the Histogel solution. Make sure you transport Histogel in 65°C water in a beaker. (Histogel can be aliquoted in to smaller volumes to avoid heating and thawing as it is recommended to use the 10ml vial of Histogel within a week once thawed.)

1. Take out the 4-well chamber slide from the incubator and aspirate the culture media in two stages – first using a P1000 tip to remove most of the media, second using P200 tip to remove the remaining media. Be careful not to touch/disturb the Matrigel bed or the organoids. It would be safe to leave few microliters of media behind to be on the safe side of not disturbing the structures. Keep in mind, it is very easy to aspirate off organoids and end up having nothing to proceed forward.
2. Wash the wells twice by gently, drop wise, adding 400 µl of Phosphate Buffered Saline (PBS). Aspirate PBS immediately after addition and no need to incubate.



3. Add 800 μl of 4% PFA per well of a 4 well chamber slide or 400 μl per well of a 8 well chamber slide and incubate for 2.0 hours at room temperature in a fume hood. Do not place it on a rocker or any moving platform as it will disturb the Matrigel layer. (Note: Adding cold PFA can melt the Matrigel it would be advisable to use PFA at room temperature). While the fixation is in progress, complete steps 4 and 5.
4. Place the Cryomolds on ice.
5. Bring the Histogel from 65°C waterbath in a beaker with water at 65°C. Add 150 μl histogel into 1 cryomold (10x10x5mm) and gently spread it with a pipet tip. Place the Cryomold back on ice for 5 -10 minutes.
6. Bring back the Histogel tube to water bath.
7. Aspirate the PFA in two stages as described above.
8. Wash 3 times with ddH₂O, 400 μl each. Keep in mind to be gentle with both addition and aspiration of ddH₂O in order to not disturb the Matrigel layer or the organoids themselves.
9. Break the chamber slide carefully. Blot the slide gently with a Kim wipe to remove excess water
10. Using a .22 surgical blade, gently scrape off the Matrigel and transfer into the Cryomold with histogel base. Allow the Matrigel to fall as a blob and do not spread it. Take care not to scrape the blue paint on the chamber slide. (Can transfer upto 4 wells of 8 well chamber slide or 2 wells of a 4 well chamber into 1 mold).
11. Add 150 μl Histogel (from the 65°C waterbath) on top of the Matrigel in the Cryomold forming a sandwich (see below)



12. Cool on ice for 10 -15 minutes.
13. To transfer the histogel block into a tissue cassette, use a .11 surgical blade to gently release the gel from the mold by running the blade along the gel-cryomold border on opposite sides. Do not release on all four sides as it may result in breaking of the histogel block. Gently flip the histogel into a tissue cassette (see below).



14. Place the tissue cassette in 10% Formalin for 16-20 hours.
Note : PFA does not fix histogel as well as formalin.
15. Transfer the cassette to a container with 0.5% Eosin in 70% ethanol for 1 – 2 days
16. After 2 days, store the cassettes in 70% ethanol until they can be processed by the histopathology core for paraffin embedding.

Recipe

4% Paraformaldehyde solution (100 ml): Heat 80 ml phosphate Buffered saline to $\sim 60^{\circ}\text{C}$ in a ventilated hood on hot, stir plate. Add 4.0 grams paraformaldehyde powder and stir maintaining the temperature higher than 60°C . Do not let the solution boil. Slowly raise the pH using 1N NaOH until the solution clears. Once cooled, adjust the volume to 100 ml.