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Fixation/Dehydration Protocol for Organoids

Morrisey Lab[1]

[1]University of Pennsylvania

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LungMap2 Consortium
Tech. support email: **lungmap2dcc@gmail.com**
Click here to message tech. support

Chelseahortman

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ABSTRACT

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ABSTRACT

Fixation/Dehydration Protocol for Organoids

Fixation/Dehydration Protocol for Organoids:

Materials:
2% PFA70% etOHrazor blade
1xPBS95% etOH10mL serological pipette
30% etOH100%etOHpipette-man
50% etOHP1000 pipette/tips24-well plates
Histogeltimer

whenever taking down/dehydrating organoids, take pictures on the Smilow EVOS/iPS Nikon of the organoids using the brightfield before starting; save images with Lung ID, age of organoids (d16, d21, etc), media conditions, and date

In Histogel:

1. Allow 50mL aliquots of 2% PFA, 1xPBS, 30% etOH, 50% etOH, 70% etOH, 95% etOH to room temp.
2. Warm histogel **(VWR cat #** 83009-992) in 65C water bath until liquid.
3. Place parafilm over a 12 well plate, making a divot in each well.
4. Using a scalpel, cut the plastic of the bottom of the transwell away. Use forceps to peel the plastic away and use the scalpel to

scrape the matrigel onto the parafilm. If the matrigel sticks to the transwell, cut a p1000 tip in half with a razorblade and use that to pop out the matrigel plug onto the parafilm.
5. Repeat with each transwell. Organoids in the same condition can all be put in the same divot (for example, all SAGM HT2-280+ can go together, all CHIR HT2-280- EpCAM+ can go together, etc.)
6. Take histogel out of 65 bath and dispense over the organoids, using a P1000, so they are completely embedded (approximately 200uL).
7. Place plate in 4C for 5-10 minutes to allow histogel to harden.
8. Transfer embedded histogel plugs from parafilm to a 12 well plate.
9. Follow the table below to complete the fixation and dehydration steps.

2% PFA @4C	overnight
1xPBS x 3 @RT	15 min
30% etOH @RT	30 min
50% etOH @RT	30 min
70% etOH @RT	30 min
95% etOH @RT	30 minx2 or 1 hour
100% store @ -20	1-4 days

Note**Fixation is done at 4C. The PBS washes and dehydration is done at room temperature. For a 12 well plate, use 1 mL of liquid per well.

Non-Histogel

1. Bring 50mL aliquots of 2% PFA, 1xPBS, 30% etOH, 50% etOH, 70% etOH, 95% etOH to room temp.
2. Get 24-well plates, labeling them to match the original organoid plate. (The 24-well plates are what you will do the fixation/dehydration in)
3. Follow the table below to complete the fixation and dehydration steps. (This entire process is done at room temp)

2% PFA	30 min
1xPBS x 3	15 min
30% etOH	30 min
50% etOH	30 min
70% etOH	30 min
95% etOH	30 minx2 or 1 hour
100% store @ -20	1-4 days

1. For the first step, add 1 mL of 2%PFA to each well.
2. Insert the transwell of the corresponding well into the new well with 2%PFA.
3. Add 600uls of 2%PFA to the transwell and let incubate for 30 min @ room temp.

(While waiting, prepare another 24-well plate with PBS, adding 1 mL to the wells)

1. Using a P1000, extract 300uls of 2%PFA from the transwell, making sure to take from the top, while being careful to not disrupt the matrigel that sits on the bottom.
2. Insert the transwell of the corresponding well into the new well with PBS.
3. Add 400uls of PBS to the transwell and let incubate for 15 min @ room temp.
4. Repeat the PBS wash two more times.
5. After finishing 3- PBS washes, follow the table completing the dehydration process (30% etOH--->100% etOH), making sure to add 1mL to the well and 400uls to the transwell.
6. After completing all steps wrap plate in parafilm and store in *-20°*freezer.

Note** We noticed when imaging the organoids that some matrigel would dislodge or disintegrate in the transwell during the dehydration process. Therefore, it is best to use the histogel to better preserve the organoids while fixing/dehydrating.