University of Arizona Cancer Center
Room 0917 Phone (520) 626-7319
UACC-TACMASS@uacc.arizona.edu

TACMASR OPERATING PROCEDURE

SOP #0	Subject	Sheet 1 of 1

Rev 0		Effective Date	Author
	Collecting Organoids and placing into Histogel Protocol	06/12/2020	BW

1. **PURPOSE: To collect, store and embed organoids**

2. **SCOPE: Collect, embed or long-term storage of organoids**

3. **RESPONSIBILITIES: Properly collect, store and embed organoids**

4. **MATERIALS and EQUIPMENT: Organoids, pipette, cold PBS, tubes, centrifuge, paraformaldehyde, Histogel, ice, PPE**

5. **PROCEDURE:**
   - **#1. Collect attached/desired cells by removing media and covering in cold PBS to dissolve Matrigel. Harvest cells and PBS into tube(s), repeat process swishing around the well to ensure all cells have been collected.**
   - **#2. Centrifuge tube(s) for 5 minutes @ 400 g (1200 RPM's)**
   - **#3. Remove PBS from tube(s) carefully, leaving organoids that have collected near bottom of tube(s). Leaving a tiny bit of PBS is OK.**
   - **#4. Fix in 3.7% Paraformaldehyde (room Temp) for 20 minutes.**
   - **#5. Centrifuge tube(s) for 5 minutes @ 400 g (1200 RPM's)**
   - **#6. Remove Paraformaldehyde then add 200-300 ml Histogel, suspend cells throughout the Histogel by gently sucking them up and releasing them throughout the gel a few times. Place on ice for at least 20 minutes or until the Histogel is set and no longer in a liquid state. (I have left on ice in the refrigerator overnight).**
   - **#7. Long term storage can be done by harvesting, fixing and storing in PBS in refrigerator. The sample should be centrifuged after storage and before removing PBS.**
   - **#8. Send to lab on ice for process and embedding**
6. **Safety and Cautionary Notes:**
   Once melted histogel should stay at approximately 60-degrees Celsius. It will degrade with time and evaporate. To the best of our Knowledge smaller amounts of Histogel can be removed from tube and melted for use thus saving the rest in the refrigerator for future use

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