

Fixation and imaging for MitoTimer TG mouse tissues.v1

1. Harvest tissues and cut them into 1-2 mm strips.
2. Fix the samples in 1 ml of ice-cold 4% paraformaldehyde at 4°C with rocking for 20 min.
3. Transfer the samples to 5-10 ml ice-cold 15% sucrose in PBS and rock at 4°C until the sample sinks to the bottom of tubes (takes about 10 min).
4. Transfer the samples to 5-10 ml ice-cold 30% sucrose in PBS and rock at 4°C until the sample sinks to the bottom of tubes (takes about 30 min).
5. Blot the sample on Kimwipe and freeze in OCT pre-chilled in freezing isopentane.
6. Section the samples and place them on slides.
7. Incubate the samples with 200 µl of 1 µg/ml DAPI at RT for 10 min on the same day or next day.
8. Wash in PBS for 5 min x 4, WIPE.
9. Mount the slide with coverslip using VECTASHIELD mounting media.
10. Acquire confocal images immediately using the parameters as described in JBC paper.