MOLab Optimized Decalcification Protocol for Decelleularized Bone Matrix (DBM) (or Bone Disc) Scaffolds

(Meng Zhang @ 03/12/2020; edited by TCH)

1) Fix the retrieved DBM masses in 5-10 volumes of 10% PBS buffered formalin for 12-24h.
2) Wash/rinse all fixed samples in slowly running tap water for 30-60 minutes. Avoid rinsing in rapidly running water.
3) [**Optional**: You can do microCT imaging at this stage].
4) Decalcify samples in ~20 volumes of **10% nitric acid** for **5~7h at 37 °C** water bath with gentle agitations [**Note**: you should check the decal status every 30min after 5h to avoid excessive decalcification in nitric acid].
5) [**Optional:** Rinse samples in slowly running tap water for 30-60min; leave samples in our regular Cal-Ex II decal-fix solution for another 12-24h].
6) Once the decalcification is complete, rinse samples in running tap water thoroughly overnight, followed by paraffin embedding using the automatic tissue processor in J-337.

NOTES:

1) Samples should not be clustered together nor sit at the bottom of container in order to provide for complete decalcification;
2) To check the end-point of decalcification, simply insert a pin/syringe needle to see if they are easy to be pierced.