

Frozen Tissue for ChIP_ Cross Linking Homogenized Sample v 1.2

Note: as a guide, plan on 50mg samples per ChIP

1. Remove pulverized tissue from freezer to bucket containing dry ice.
2. Invert bag and tap frozen material into 5 ml Eppendorf LoBind tube cat. # 223100034 and place tube in wet ice.
3. Use 1 ml room temperature PBS with 2x protease inhibitors at a time to clear additional tissue from Kapton tissue tube. Do not exceed 4 ml.
4. Place additional material in the 5ml tube on ice.
5. Re suspend tissue with wide orifice tip. Be careful to remove your entire sample from pipet tip.
Note* if you are working with sticky samples, just invert tube to mix sample.
6. Cross link tissue in (RT) PBS with protease inhibitors to a final concentration of 1% formaldehyde.
(Add 62.5 μ l of 16% methanol free formaldehyde per 1 mL solution)
7. Incubate at RT for 15' with gentle agitation on a rotator or rocker.
8. Quench crosslinking by adding fresh 2.5M glycine to a final concentration of 0.125M.
(Assuming previous vol. was 1062.5 μ l per mL; add 53.125 μ l per mL of 2.5M Glycine)
9. Incubate at RT for 5' w/ gentle agitation, then place on ice.
10. Centrifuge at 3500 rpm, 5', 4°C and aspirate supt.
(if your tissue is fatty, increase speed of centrifugation to adequately pellet material)
11. Wash with 4 mL of 4°C PBS w/ 2x protease inhibitors per tube.
12. Centrifuge at 3500 rpm, 5', 4°C and aspirate supt.
13. Resuspend cross-linked tissue in 4°C PBS w 2x protease inhibitors and aliquot material as evenly as possible into 1.5 mL eppendorf tubes so that each tube represents approximately 50mg of cross-linked material.
14. Centrifuge at 3500 rpm, 5', 4°C and aspirate supt.
15. If pellets are not equal in size, you can make adjustments using additional 4°C PBS with 2x protease inhibitors
16. * You can snap freeze your cross linked material and store at -80°C or proceed with lysis and shearing.

