THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Simplified Acid Histone Extraction
Optimized for the Fisher Scientific Sonicator Model 500.

-Harvest and pellet cells, wash twice with cold PBS, make sure you have at least 40-50 uL of packed cells, in order to have a good yield of histones.
If using 293 cells, one confluent 10-cm plate should be more than enough.

-Lyse cells in Ripa buffer with appropriate Protease inhibitors, Do not sonicate the sample at this time. If cyto and nuclear extracts are needed, use the NE-PER kit from Pierce.

-After whole or fractionated lysis is complete, centrifuge the sample at maximum speed for 10 min, and transfer the supernatant in a fresh tube.

-Now, you will work on the pellet, which contains the chromatin fraction. Add 150 uL of 0.8 M HCl. You will notice that the pellet turns more white. Sonicate at 30% power for 15 seconds (this is a harsh sonication). Leave the sample on ice for 1 hr. At this point, the histones are freed from the chromatin.

-Centrifuge the sample at maximum speed for 10 minutes. Transfer the supernatant in a fresh tube.

-Neutralize the sample by adding Tris –HCl 1.5 M pH 8.5. Add **90 uL** of this solution to the sample. Generally speaking, this solution may not be enough to completely neutralize the sample, but is better to not go to a further point where the samples became too basic (because the running will not be nice).

Take 10 uL of this sample, add LB. If the sample turns yellow at this point, you need to add a bit more of neutralizing solution to it. Add 0.5 uL. If the sample does not turn blue, add another 0.5 uL and so on until it turns blue.

Load the histones on a coomassie gel for quantification.