

Supplemental Protocol: Yeast Lysis with PIXUL™ Multi-Sample Sonicator

Description: PIXUL Multi-Sample Sonicator makes yeast lysis easy.

IMPORTANT: PIXUL sonication requires using PIXUL 96-well round bottom plates (cat. no. 53139).

1. Add 500 μ l 5% SDS 50 mM TEAB, and measure pH which should be 7-8. Buffer volume can be less for smaller pellets.
2. Load 100 μ l sample in each well of 96-well microplate, seal, and sonicate in PIXUL for 15-30 minutes (min).
3. PIXUL parameters: ulse Pulse N=50, PRF=1 kHz, Process Time = 30 or 60 minutes, Burst Rate=20 Hz.
4. Quick-spin 96-well microplate. Transfer sonicated lysates into centrifuge tubes. Go to step 9.
5. Or, centrifuge 96-well microplate at maximum speed for 15 minutes, extract only supernatant. Go to step 10.
6. To compare PIXUL in parallel with probe sonicator, after step 1, transfer lysate of 3 tubes to centrifuge tube with lysing matrix Y.
7. Lyse in Qiagen tissue lyser for 5 min, 30 Hz. *Keep aside 10 μ l aliquot for agarose gel.*
8. Perform bead beating followed by probe sonication: 3 cycles x 10 seconds, 30s on ice in between cycles.
9. Centrifuge for 15 min at maximum speed i.e. 13.2k rpm.
10. *Keep aside 10 μ l aliquot for agarose gel.*
11. Perform BCA assay.
 - a. Dilute sample 16x (2.5 μ l sample + 37.5 μ l 5% SDS 50 mM TEAB)
 - b. Use 10 μ l of diluted sample per replicate in 200 μ l WR (50:1, A:B)
12. Quantify protein extraction in μ g, isolate 100 μ g protein extract.
13. Adjust volume to 50 μ l and 5% SDS 50 mM TEAB f.c.
14. Add 1.5 μ l 0.5 M DTT, 30 min @55°C.
15. Add 3 μ l 0.5 M IAA, 15 min @RT in dark.
16. Add 5 μ l 12% phosphoric acid, check pH<1. If pH>1, add more phosphoric acid.
17. Add 350 μ l 90% MeOH 100 mM TEAB pH 7.55.
18. Pipet samples on S-trap plate, centrifuge for 2 min at 1500xg.
19. Wash 3 times with 200 μ l 90% MeOH 100 mM TEAB pH 7.55, centrifuge for 2 min at 1500xg.
20. Add 125 μ l 50 mM TEAB containing 1 μ g Trypsin (1:100).
21. Overnight incubation in 37°C, cover loosely with the cover supplied with S-trap plates.
22. Add 80 μ l 50 mM TEAB, centrifuge for 2 min at 1500xg.
23. Add 80 μ l 0.2% formic acid, centrifuge for 2 min at 1500xg.
24. Add 80 μ l 50% ACN, 0.2% formic acid, centrifuge for 2 min at 1500xg.
25. Transfer to MS vials, speedvac and store at -20°C.

Protocol courtesy of Vlaams Instituut voor Biotechnologie (VIB), Belgium; VIB Proteomics Core

Technical Support

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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