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 µl 5% SDS 50 mM TEAB to yeast pellets derived from 2 ml overnight cultures. Measure pH which should be 7-8. Buffer volume can be less for smaller pellets.
2. Load 100 µl 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 µl 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 µl aliquot for agarose gel.*
11. Perform BCA assay.
    a. Dilute sample 16x (2.5 µl sample + 37.5 µl 5% SDS 50 mM TEAB)
    b. Use 10 µl of diluted sample per replicate in 200 µl WR (50:1, A:B)
12. Quantify protein extraction in µg, isolate 100 µg protein extract.
13. Adjust volume to 50 µl and 5% SDS 50 mM TEAB f.c.
14. Add 1.5 µl 0.5 M DTT, 30 min @55°C.
15. Add 3 µl 0.5 M IAA, 15 min @RT in dark.
16. Add 5 µl 12% phosphoric acid, check pH<1. If pH>1, add more phosphoric acid.
17. Add 350 µl 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 µl 90% MeOH 100 mM TEAB pH 7.55, centrifuge for 2 min at 1500xg.
20. Add 125 µl 50 mM TEAB containing 1 µg Trypsin (1:100).
21. Overnight incubation in 37°C, cover loosely with the cover supplied with S-trap plates.
22. Add 80 µl 50 mM TEAB, centrifuge for 2 min at 1500xg.
23. Add 80 µl 0.2% formic acid, centrifuge for 2 min at 1500xg.
24. Add 80 µl 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.

Active Motif North America

1914 Palomar Oaks Way, Suite 150
Carlsbad, CA 92008, USA

Toll Free: 877 222 9543
Direct: 760 431 1263
Fax: 760 431 1351
E-mail: tech_service@activemotif.com

Active Motif Europe

Waterloo Atrium
Dréve Richelle 167 – boîte 4
BE-1410 Waterloo, Belgium

Germany Free Phone: 0800 181 99 10
France Free Phone: 0800 90 99 79
UK Free Phone: 0800 169 31 47
Fax: +32 (0)2 653 0050
E-mail: eurotech@activemotif.com

Active Motif Japan

Azuma Bldg, 7th Floor
2-21 Ageba-Cho, Shinjuku-Ku
Tokyo, 162-0824, Japan

Direct: +81 (0)3 5225 3638
Fax: +81 (0)3 5261 8733
E-mail: japantech@activemotif.com

Active Motif China

290 Wankang Road
Suite 601
Minhang District
Shanghai, 201112, China

Direct: (86)-21-20926090
Cell Phone: 18521362870
E-mail: techchina@activemotif.com

Visit Active Motif online at http://www.activemotif.com