Nucleus Protocols

Lyse Bacteria by Sonication

☐ **Prepare Stock Solutions.**

Reagent	MW (g/mol)	Amount (g)	Final Volume (mL)	Storage	Needs pH adjustment?	Needs Sterilization?
Potassium Hydroxide (1M)	56.11	14.0	250	4 °C to 30 °C	no	no
HEPES-KOH (pH=7.6; 1M)	238.3	59.5	250	4 °C to 30 °C; dark	yes	no
Ammonium Chloride (1M)	53.49	13.4	250	4 °C to 30 °C	no	no
Sodium Chloride (5M)	58.44	73.1	250	4 °C to 30 °C	no	no
Magnesium Chloride (1M)	203.3	20.3	100	4 °C to 30 °C	no	no
Lysozyme (30 mg/mL)	14320	1	33	−25 °C to −15 °C	no	no

☐ **Prepare Lysis Buffer** — used to resuspend bacterial pellets for mechanical lysis by sonication. Also chemically lyses cells with lysozyme (*G. gallus*). Contains protease inhibitor (cOmplete) to slow proteolysis. *Make fresh and use the same day!*

Reagent	Final Concentration (mM)	Stock Concentration (mM)	Volume to Add (mL)
HEPES-KOH	50	1000	5
Ammonium Chloride	100	1000	10
Sodium Chloride	500	5000	10
Magnesium Chloride	10	1000	1
Lysozyme	0.3 mg/mL	30 mg/mL	1
TCEP	1	500	0.2
cOmplete Protease Inhibitor	1 tablet / 50 mL	n/a	2 tablets
water (milliQ)	—	—	72.8
Total	—	—	100

☐ **Prepare centrifuge and Lysis Buffer.**

- ☐ Pre-chill your centrifuge and Lysis Buffer to 4 °C.
- ☐ Finish Lysis Buffer with TCEP (0.5M) (e.g., add 500 µL TCEP to 1 L Lysis Buffer).

☐ **Resuspend pellets in cold Lysis Buffer.**

- ☐ Thaw bacterial pellets on ice.
- ☐ Weigh 1 g dry cell mass per pellet.
- ☐ Resuspend 1 g pellets in 10 mL Lysis Buffer by vortexing.
- ☐ Keep lysate cold to prevent proteolysis.

- ☐ **Lyse cells by sonication.**
    - ☐ Program your sonicator. We recommend 50% amplitude, 10 s on / 10 s off.
    - ☐ Always use a Sound Enclosure during sonication.
    - ☐ Place resuspended samples under the sonicator horn in an ice bucket on a lab jack.
    - ☐ Adjust your sample height using the sample jack until each sonicator tip is submerged by about one quarter (1/4) of the sample height (e.g., 2.5 mL into a 10 mL sample).
    - ☐ Lyse cells by sonication. Deliver 4.5 kJ for every 10 mL sample (e.g., a four-pronged sonicator horn lysing 4x 10 mL samples arrow.r 18 kJ).
    - ☐ Avoid foaming during sonication.
- ☐ **Clarify lysates by centrifugation and filtration.**
    - ☐ Centrifuge lysates at 4 krcf / 4 °C / 45 min.
    - ☐ (Meanwhile) for each lysate, assemble a 10 mL Luer lock syringe and 0.22 µm syringe filter.
    - ☐ Carefully decant each supernatant into a syringe and filter into a fresh 15 mL centrifuge tube. Keep clarified lysate cold (4 °C).
    - ☐ Keep going! Don’t take a break!