Adapted from Voss et al., J Biol Chem. 2002 May 17;277(20):17493-501

Nuclear protein extraction from *Plasmodium falciparum*

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Buffers, centrifuges, rotors, tubes, and so on should be kept at 4°C to reduce degradation of proteins from protease activity.

Reagents

- **PBS**
- **fresh 10% saponin solution to make 0.1% saponin/PBS solution**
- **Buffers**
  - **§ Lysis buffer:** 20 mM HEPES, pH 7.8; 10 mM KCl; 1 mM EDTA; 1 mM DTT; 1 mM PMSF; 1% Triton X-100
  - **§ Extraction buffer:** 20 mM HEPES, pH 7.8; 800 mM KCl; 1 mM EDTA; 1 mM DTT; 1 mM PMSF; 1x protease inhibitor cocktail (Sigma product # P1860-1ML)
  - **§ Dilution buffer:** 20 mM HEPES, pH 7.8; 1 mM EDTA; 1 mM DTT; 30% glycerol

  prepare a small volume of 100 mM DTT in ethanol and 100 mM PMSF in Isopropanol (both have short shelf life) prior to extraction, dilute both to 1 mM in the buffer volume needed for extraction procedure;
  add protease inhibitor prior to use to make 1x

Materials

- 50 mL conical tubes
- 1.5 mL and 2 mL microcentrifuge tubes
- bench-top centrifuge (Beckmann Coulter Allegra™6R, swinging bucket rotor GH-3.8) cooled to 4°C
- microcentrifuge (eppendorf centrifuge 5415D, rotor F45-24-11) cooled to 4°C
- Laminar air flow cabinet
- pasteur pipette attached to vacuum line
- rotator (Clay Adams® Brand Nutator # 421105) at 4°C

A. Culture Preparation

1. Transfer parasite cultures, 3 or 4 50 mL cultures, into 50 mL centrifugation tubes. Harvest cells at 1500 rpm (800xg) 5min, low brake.
2. Aspirate the supernatant.
3. Resuspend the pellets in 5 mL PBS and and pool into one 50 mL conical tube. Fill up to 50 mL with PBS. Centrifuge at 1500 rpm (700xg) for 5min. Remove the supernatant.

B. Red Blood Cell Lysis

4. Resuspend pellet in 50 mL 0.1% PBS/saponin solution.
5. Incubate 10 min on ice.
6. Centrifuge at 3750 rpm (2000xg) for 10 min. Aspirate the supernatant.
7. Resuspend pellet in 20 mL of 0.1% PBS/saponin and spin at 800g for 10min, low brake.
8. Repeat washing step 7 with 20 mL PBS.

C. Parasite Lysis

9. Resuspend the parasite pellet in 2 mL lysis buffer, transfer to 2 mL microcentrifugation tube and incubate on ice for 5min.
10. Pellet nuclei at 5200 rpm (2500xg) for 5min at 4°C.
11. Aspirate the supernatant and wash twice with 1 mL lysis buffer.

D. Protein Extraction

12. Add 1 to 2 pellet volumes of extraction buffer.
13. Incubate for 30 min while rotating at 4°C.
14. Clear the extract by centrifugation at 11800 rpm (13000xg) for 30min at 4°C.

E. Storage of extracted proteins

15. Transfer the supernatant containing nuclear proteins to a fresh 1.5 mL microcentrifugation tube and dilute with 1 volume of dilution buffer.
16. Store at -80°C.