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Protein Sample Preparation for Proteomic Analysis in *Leishmania donovani*

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[Abstract] *Leishmania* is a genus of trypanosomatid protozoa and is the parasite responsible for the disease leishmaniasis. These protozoa, regulate their gene expression in an atypical way, compared to other higher eukaryotes. The regulation of gene expression is characterized by a predominance of post-transcriptional over pre-transcriptional regulatory mechanisms (Clayton, 2002). Thus proteomic analysis has proven an essential tool for understanding pathways implicated in *Leishmania* infectivity, host-parasite interactions, drug resistance and others. When employing a comparative proteomics analysis between different parasitic cell lines, it is essential that these lines are cultivated in exactly the same way, in the same cell density and growth phase. More importantly when cell-cycle defects are suspected, it is essential to synchronize cell-lines in the same cell-cycle phase so as to eliminate possible artifacts. This protocol describes the preparation of whole-protein samples for proteomic analysis in *Leishmania donovani* (*L. donovani*).

Materials and Reagents

1. *Leishmania donovani* (*L. donovani*) parasites
2. Glucose (Sigma-Aldrich, catalog number: G8270)
3. Hydroxyurea (Sigma-Aldrich, catalog number: H8627)
4. RPMI-1640 (Life Technologies, catalog number: 21875-034)
5. Fetal Bovine Serum heat inactivated (HIFBS) (Life Technologies, catalog number: 10270-106)
6. Penicillin/Streptomycin (Life Technologies, catalog number: 15140-122)
7. HEPES buffer (Life Technologies, catalog number: 15630-056)
8. Dry ice
9. Ethanol
10. E-64 (Sigma-Aldrich, catalog number: E3132)
11. CHAPS (Sigma-Aldrich, catalog number: 26680)
12. ASB-14 (Sigma-Aldrich, catalog number: A1346)
13. DTT (Sigma-Aldrich, catalog number: 43815)
14. Bio-Lyte® 3/10 Ampholyte (Bio-Rad Laboratories, catalog number: 163-2094)
15. Urea (Sigma-Aldrich, catalog number: U5378)
16. Thiourea (Sigma-Aldrich, catalog number: T7875)
17. Bromophenol blue (Bio-Rad Laboratories, catalog number: 161-0404)

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18. Phosphate Buffered Saline (PBS) (see Recipes)
19. Lysis Buffer (see Recipes)
20. Rehydration Buffer (see Recipes)
21. Fully supplemented medium (see Recipes)

Equipment

1. Pipette
2. Centrifuge
3. Spiramix

Procedure

1. *Leishmania donovani* parasites in the logarithmic phase (~5 x 10^6 parasites/ml) were cultured in fully supplemented culture medium at 25 °C and synchronized with the addition of 2.5 mM hydroxyurea for 12 h.
2. After this period of time the parasites are gently washed three times with 10 ml of PBS and released in 10 ml of fully supplemented RPMI medium.
3. 5 x 10^8-10^9 parasites are harvested by centrifugation (500 *x g*, 15 min, RT)
4. The parasitic pellet is washed twice with 10 ml of PBS supplemented with 0.1% (w/v) glucose.
5. The parasitic pellet is resuspended in 50 µl Lysis buffer using a pipette.
6. The lysed parasites are subjected to five freeze-thaw cycles in solid CO_2/ethanol.
7. The sample is centrifuged at 20,000 *x g* for 30 min at 4 °C.
8. To the supernatant 400 µl of Rehydration buffer [containing 0.2% (v/v) Bio-Lyte®] are added.
9. The sample is incubated for 30 min at RT under gentle rotation.
10. After the end of the incubation, the sample is centrifuged at 17,000 *x g* for 40 min at RT.
11. The supernatant can now be used for proteomic analysis or can be stored at -80 °C for future use.

Notes

1. Extra caution is required at all steps of the procedure as not the protein sample to be contaminated by environmental proteins (*e.g.* keratin). Preferably avoid using bare hands during the procedure.
2. After the addition of Rehydration buffer the sample should not be exposed to temperatures below 10 °C, due to urea-mediated sedimentation.
3. Bio-Lyte® should be added just before the use of the Rehydration Buffer.

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Recipes

1. PBS
   Dissolve
   8 g of NaCl
   0.2 g of KCl
   1.44 g of Na_2HPO_4
   0.24 g of KH_2PO_4
   In 800 ml distilled H_2O
   Adjust pH to 7.4
   Add H_2O to 1 L
   Sterilized by autoclaving
2. Lysis Buffer
   400 µl Tris-HCl (pH 9.5)
   20 µl 0.5 M EDTA (pH 8.0)
   100 µl 1 mM E-64
   Add distilled H_2O up to 10 ml
   Aliquot and stored at -20 °C
3. Rehydration Buffer
   To a volume of 3 ml distilled H_2O dissolve
   4.8 g urea
   1.52 g thiourea
   0.4 g CHAPS
   0.1 g DTT
   0.24 g ASB-14
   5 µl 0.1% (w/v) bromophenol blue
   Add distilled H_2O up to 10 ml
   Aliquot and stored at -20 °C
4. Fully supplemented medium
   RPMI-1640
   10% (v/v) HIFBS
   10 mM HEPES
   100 U/ml Penicillin/Streptomycin

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