

INVESTIGATION OF A PROTEIN EXPRESSION PROFILE BY HIGH
RESOLUTION BIDIMENSIONAL ELECTROPHORESIS OF *TRYPANOSOMA*
CRUZI EPIMASTIGOTES

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Trypanosoma cruzi has a complex life cycle with several developmental stages. The epimastigote form, a parasite life stage encountered in the gut of triatomine insect vectors, has to adjust to nutrient availability in this environment. Here we present the standardization of high resolution, reproducible and sensitive bi-dimensional (2D) gels of soluble protein fractions. The main parameters considered for 2D gel standardization were: fractionation method, sample treatment and the choice of pH range for first dimension separation. Parasites were cultivated in protein free defined AR103 medium and soluble proteins were used to evaluate two different sample treatment protocols for 2D electrophoresis. The results show that the improvements obtained in the resolution and reproducibility of our 2D gels in the improved protocol can be mainly attributed to the reduction in the percentage of TCA, increased incubation time for protein precipitation and alkylation treatment, and by the increased concentration of alkylation agent. Thus far 56 protein spots were identified by MALDI-TOF/TOF spectrometry. This method yields high resolution, reproducible and sensitive 2D gels of soluble protein fractions and has proved useful in the study of the differentially expressed protein profile of *T. cruzi* epimastigote forms cultivated in defined media and metabolic conditions, or challenged with drugs.
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