11.67

EXTRACTION AND PURIFICATION OF MYOSIN FROM HUMAN CARDIAC MUSCLE

T.H.O. Lohmann*, K. Okazaki and J.R. Rogero

Divisão de Radiobiologia - TBR

Comissão Nacional de Energia Nuclear - IPEN-CNEN/SP

The aim of the present project was to improve the method for the human myosin extraction and purification to use it as antigen to obtain monoclonal antibody. The antimyosin will be cloned to be used in imaging of experimental myocardial infarction and some others experiments in Nuclear Medicine.

The methods of Sarkis et al[1] has permited to define rabbit skeletal muscle myosin qualitively and quantitively. We tested this method to adequate it for the extraction of myosin from human cardiac muscle. The human myosin will be employ it in the future in isogenic mice immunization.

The extraction was carried out with dilutions in phosphate buffers, KCl and EDTA pH6.5 in different concentrations. The protein content of each step of extration was determined by Bradford (1976) method. The myosin solution was purified by ionic exchange chromatography on DEAE-sephadex A-50 equilibrated in phosphate 0,15M and EDTA 0,01M buffer with a 0-1M KCl gradient. The myosin obtained was identified in PAGE eletrophorese. The material obtained was stored by liofilization with glicin. The result of purification was 28,13 mg/200g cardiac muscle. The method showed to be feaseable also for the purification of cardiac muscle.

1-Sarkis S. Margossian and Susan Lowey. Methods in Enzymology,85: 55-71, 1982.