

Detection of myofibrillar proteins using a step gradient minigel with an ambiguous interface

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摘要

Abstract

Myosin heavy chain (MHC), actin, titin, and nebulin are four major myofibrillar proteins that interact with each other. However, it is difficult to analyze the four proteins simultaneously on the same minigel due to their broad range of molecular weights. Numerous gradient gels are normally used to detect these myofibrillar proteins. The conventional step gradient gel provides better separation of the four major proteins, but several proteins accumulate at the interfaces between different gradient layers. To eliminate the obvious interfaces, we employed a plastic syringe filled with 12 and 4% acrylamide solutions simultaneously and then established an improved step gradient minigel with an ambiguous interface. It was determined by blue dextran in-gel visualization and scanning densitometry that the acrylamide concentration at the ambiguous interface gradually changed. Coomassie blue staining and immunoblotting revealed that the four proteins were successfully separated and transferred for analysis. This gel system is simple to prepare and easy to use, and it is a reliable method for analyzing myofibrillar proteins or other protein mixtures with broad molecular masses.