MLCK Purification

Fresh rabbit fast skeletal muscle was isolated from a female New Zealand white rabbit euthanized using the captive bolt gun method followed by exsanguination according to a UM-ACUC approved protocol. Muscle was cleaned, cut and chilled for 5 minutes on ice before being placed for 5-10 minutes at 20^{o}C. After the incubation, muscle was minced in a pre-chilled meat grinder that had previously been treated with 2mM EDTA and rinsed with ice cold double distilled water. The muscle mince was then extracted on ice for 15-20 minutes in ice cold Guba-Straub buffer (0.1M KH_2PO_4, 0.05M K_2HPO_4, 0.3M KCl, pH 6.5) (1.5L/500g) muscle with stirring. The muscle was then centrifuged at 11,000 x g $_{avg}$ for 30 minutes at 4^{o}C. The supernatant was decanted through glass wool to remove fat and the pellet discarded. The myosin containing supernatant was then precipitated with 13-15 volumes of ice cold 10mM phosphate buffer, pH 7. The myosin was centrifuged as above and then dissolved in a minimal volume of room temperature 0.5M KCl, 20mM phosphate buffer, pH 8, 12.5mM MgCl2, and 0.1mM CaCl2. ATP was then added to a final concentration of 5mM and the myosin was incubated at room temperature for 30 minutes to activate the MLCK. The myosin was then subjected to a 0-42.5% ammonium sulfate precipitation done slowly with stirring on ice while maintaining a constant pH (6.5-7). The mixture was next centrifuged at 11,000 x g $_{avg}$ for 10 minutes at 4^{o}C. Pellets obtained from the first ammonium sulfate cut were discarded and the retained supernatant subjected to a further 42.5-55% ammonium sulfate$AmSO_4$ precipitation. After centrifugation as previously, the supernatant was discarded and the pellet was resuspended in a minimal volume of 40mM KCl, 2mM $MgCl_2$, 10mM Imidazole, pH 6.6, 10mM Bis-Tris, 0.5mM PMSF and 0.5mM DTT. The dialysate was dialyzed 2 X 4L

against the same buffer diluted 4X. The supernatant, containing the native MLCK, was collected by centrifugation after dialysis and concentrated to a minimal volume. MLCK was aliquoted and stored frozen at -80^{o}C until used.