Burridge Laboratory Stress Fiber Isolation Protocol
(Adapted from Katoh et al., 2000)

STOCK Solutions
4X PBS
250mM TEA (triethanolamine)
20% NP-40
20% Triton X 100
Protease Inhibitors (working conc.)
1. 20 ug/ml aprotinin
2. 1 ug/ml leupeptin
3. 1 ug/ml pepstatin

WORKING Solutions (prepare fresh)
PBS + protease inhibitors (PBS)
Low ionic strength extraction buffer (LISE buffer) : 2.5mM TEA + protease inhibitors pH 8.2 (dH2O)
Extraction buffer 1 : 0.05% NP-40 + protease inhibitors pH 7.2 (PBS)
Extraction buffer 2 : 0.5% Triton X-100 + protease inhibitors pH 7.2 (PBS)

METHODS
Wash cells (Ref52 fibroblasts 15-10cm dishes) with PBS containing 1mM MgCl (MgCl is used at this step to keep the cells attached to the dish during the extraction in LISE buffer.)
Extract with LISE buffer for 20-40 minutes with 5 buffer changes; (Removes soluble components-at this point cells swell)
Extract with Extraction buffer 1 for 5 min.
(Removes dorsal surface of cells and nuclei, basal portion of cells remaining as well as some nuclei)
Extract remaining components with Extraction buffer 2 for 5 minutes.
Immediately wash with PBS to remove triton (stress fibers are unstable in triton)
Scrape off stress fibers from culture dish and suspend in small volume (<5ml) PBS
(At this point stress fibers still contain cell cortex and focal adhesion components)
Extrude 2X through Z-shaped needle; Separates free individual stress fibers from cortex
Centrifuge at 1000g for 5´ to remove large debris
Maybe higher speed to remove focal adhesion complex
Collect stress fibers by centrifugation at 100,000g for 1 hour.
Lipids were removed by Chloroform/Methanol extraction
Pellet was suspended in Ammonium Bicarbonate (AMBIC) and submited for Mass Spec.
Control stress fiber isolation to identify contaminating proteins in prep.
Ref52 fibroblasts were treated for 30 minutes with 10uM Y-27632 (Calbiochem)
Stress fibers were isolated as above.