Farkas ImmunoStain of Salivary Glands

1. Salivary glands were fixed in 4% paraformaldehyde and 1.5% glutaraldehyde in PBS

2. permeabilized with 0.3% Triton X-100 in PBS, blocked with 2% BSA and 1% normal goat serum.

3. rabbit polyclonal antibodies (1 : 300 dilution) for detecting p127 in wild-type and P-mouse monoclonal antibodies against myosin-II (1 : 20 dilution) were added and the salivary glands were incubated for 16 h at 4°C.

4. The salivary glands were washed with 0.3% Triton X-100 in PBS and blocked.

5. Cy3 goat anti-rabbit antibodies and Cy5 goat anti-mouse antibodies (Jackson Immunoresearch, Inc., West Groove, PA, USA) as well as fluoresceine conjugated phalloidin from Sigma, St Louis, MO, USA (1 : 200 dilution) were added and the glands were further incubated for 2 h at room temperature,

6. then washed,

7. mounted in Mowiol (Calbiochem, La Jolla, CA, USA). Optical sections, 1 μm thick, were collected using a Zeiss LSM-410 laser confocal microscope.