Ethidium Bromide Staining For Analysis of Cell Death during 3-D Culture

1) Remove medium from the 3-D cultures to be analyzed.
2) Prepare mixture of 1μM ethidium bromide (EtBr, Sigma) in PBS and add this solution to acini. Incubate for 15-30 minutes at 37^{o}C. If desired, 5μM calcein AM (Molecular Probes) can also be added to counterstain viable cells.
3) Remove EtBr solution and replace with PBS.
3) Analyze using indirect immunofluorescence on a microscope equipped with a mercury lamp. Ethidium bromide positive cells are readily detected on the rhodamine channel available on standard filter sets.
4) For a quantitative measure of cell death within a specific culture, the culture is stained with ethidium bromide (calcein AM should not be added in these assays) for exactly 15 minutes and the percent of acini containing one or more ethidium bromide-positive cells within a culture is counted; at least 200 structures should be counted for such quantification.