**1) Notes on cell culture of human vascular smooth muscle cells**

1. This assay may also be used with other cell types which deposit calcium crystals.
2. Typically, calcification presents as dark black speckles. Time to first occurrence may vary with chosen calcification stimulus, cell culture conditions and may be subject to hVSMC batch-to-batch variability.
3. From the first occurrence of calcification onwards, cells should be imaged daily.