GeneReleaser® Single Tube RT-PCR Protocol

1. Take an aliquot of cells (1,000,000) and transfer to PCR tube. The cell # should be such that 1-10µl will contain the 10^6 cells.
2. Add 20µl of GR and vortex.
3. Overlay with RNase free mineral oil.
4. Follow microwave protocol.
5. Add sufficient RNase free DNase to digest DNA if DNA will interfere with your PCR or RT-PCR. We use 0.1 to 1 unit of DNase per tube and incubate 1 hour at 37°C.
6. Destroy DNase by addition of 0.1 units of Proteinase K and heating at 55°C for 30 minutes. Alternatively, DNase can be heat inactivated by 94°C for 10 minutes.
7. Destroy Proteinase K activity by heating to 94°C for 10 minutes.
8. Centrifuge tubes for 2 minutes at 10,000xg to form a tight GR pellet.
9. Perform RT directly in tube limiting reagent volumes to no more than 50µl total volume. Follow manufacturer's protocol for RT.
10. Perform PCR in the tube containing the cDNA produced by RT. We recommend a 100µl PCR. Note: Adjust buffers, Mg, etc., so that the final PCR volume is 100µl and all components are 1X and RNase free.

If you have further questions, please contact us at support@bioventures.com. You may also contact us via fax (1-877-286-0330) or through our toll-free phone number (1-877-852-7846).