BIOVENTURES

cambio

GeneReleaser® Tips

We have found that certain actions are essential for the proper performance of GeneReleaser®. These are described below.

The sequence of reagents additions of the reaction Components is critical. Additions should be performed in the following order:

Use 1µl of whole blood or cells at a density of ~108/ml.

Add 20µl of GeneReleaser® (for standard 100µ amplifications).

DO NOT vortex or mix components after steps A&B above!

Lyse the cells using the thermocycle program protocol.

Add amplification reagents (DO NOT vortex or mix).

Perform amplification. NOTE: It is very important that the very first denaturing step of the first cycle be at 94°C for 2-5 minutes depending on brand of cycler, reaction volumes, etc.

The specimen and GeneReleaser® volumes may be adjusted. However, no less than 5µl of GeneReleaser® or more than 5µl of specimen should ever be used.

The volume of GeneReleaser® used to accomplish cell lysis should be compensated for by deducting an equivalent volume of H2O from the components of the amplification reagents in order to maintain their appropriate concentrations in the final reaction volume.

Amplification reaction volumes may be reduced from the typical 100µl volume to as little as 25µl as long as proportionate reductions are made with respect to specimen and GeneReleaser® volumes.

The original thermocycle program has been modified to an 80°C hold in order to obtain better amplification from denser tissue materials and to facilitate an initiation of the amplification cycles under conditions which minimize non-specific annealing of primers.

It must be emphasized that if, upon use of this product, the expected bands are not observed, then a magnesium titration should be performed. If this fails to produce the desired bands, then reduction of the annealing temperature by 5-10°C should be employed in conjunction with a magnesium titration. If either of these should fail, we will be glad to develop an optimized procedure with you.

If the GeneReleaser® treated specimens cannot be amplified after performing the procedure, specimens may be stored at either 4°C or -20°C until they can be amplified. Prior to amplification stored specimens should be heated to 80°C and the amplification begun using steps 10-12 of the GeneReleaser® protocol.