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(54) Title: RNASE H-BASED ASSAYS UTILIZING MODIFIED RNA MONOMERS

(57) Abstract: Methods and compositions are provided for improving specificity during amplification of a target DNA sequence. The methods and compositions rely upon the use of an RNase H enzyme, a polymerase, and RNase H enzyme-sensitive, blocked-cleavable oligonucleotide primers in the amplification reactions, wherein the reaction mixtures include either an optimized final concentration of a divalent metal salt comprising 2.0 mM or less of free Mg⁺⁺ cation and/or an optimized final concentration of a non-ionic detergent comprising at least about 0.001% polyethylene glycol hexadecyl ether.



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