

Guidelines for SYBR Green qPCR continued:

- Suggested input quantities of template are: cDNA corresponding to 1 pg to 100 ng of total RNA; 100 pg to 100 ng genomic DNA
- After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

Reaction Assembly

Component	Volume for 50- μ L rxn.	Final Concentration
PerfeCta SYBR Green SuperMix for iQ (2X)	25 μ L	1x
Forward primer	variable	100 – 500 nM
Reverse primer	variable	100 – 500 nM
Nuclease-free water	variable	
Template	<u>5 – 10 μL</u>	variable
Final Volume (μ L)	50 μ L	

Note: For smaller reaction volumes (i.e. 25- μ L reactions), scale all components proportionally.

Reaction Protocol

Incubate complete reaction mix in a real-time thermal detection system as follows:

Initial denaturation:	95°C, 2 to 3 min
PCR cycling (30-45 cycles:)	95°C, 10 to 15 s 55 – 65°C, 30 to 45 s (collect and analyze data)
Melt Curve (dissociation stage)	Refer to instrument instructions (optional)

Full activation of AccuStart Taq DNA polymerase occurs within 30 seconds at 95°C. Initial denaturation times greater than 3 minutes are not recommended. However, amplification of genomic DNA or supercoiled plasmid DNA targets may benefit from a prolonged initial denaturation step (5-10 min) to fully denature and fragment the template. This minimizes the potential for renaturation of long fragments and/or repetitive sequence regions that can impair replication of the target sequence by the PCR process.

Some primer sets may require a 3-step cycling protocol for optimal performance. Optimal annealing temperature and time may need to be empirically determined for any given primer set. A 68 to 72°C extension step of 30 seconds is suitable for most applications. However, amplicons greater than 200 bp may require longer extension times. The use of an elevated temperature (80°C) for data collection is not recommended. While this technique can be used to mask the detection of primer-dimer and/or other non-specific products, it does little to improve assay specificity or sensitivity and is not a substitute for effective primer design.

Quality Control

Kit components are free of contaminating DNase and RNase. PerfeCta SYBR Green SuperMix for iQ is functionally tested in qPCR. Kinetic analysis must demonstrate linear resolution over six orders of dynamic range ($r^2 > 0.995$) and a PCR efficiency $> 90\%$.

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