

## PCRboost™ Quick Reference Protocol

When using PCRboost, there is no change to your PCR or RT-PCR protocol. Simply substitute PCRboost for water.

The following are examples of PCR and RT-PCR reactions containing PCRboost. Use your own optimized protocol for specific target amplification.

### EXAMPLE: PCR with 500 bp amplicon

PCR	
Genomic DNA	pg to ng
10x buffer	5 $\mu$ l
dNTP (10 mM each)	1 $\mu$ l
Primer F (10 $\mu$ M)	1 $\mu$ l
Primer R (10 $\mu$ M)	1 $\mu$ l
DNA Taq Pol	2.5-5U
<b>PCRboost</b>	<b>bring up to 50 <math>\mu</math>l</b>
<b>FINAL VOLUME</b>	<b>50 <math>\mu</math>l</b>

PCR Cycling Protocol	
94°C	2 min
94°C	15-30 sec
55-65°C	30 sec
72°C	30 sec
72°C	4 min
4°C	$\infty$

} 32-40x

### EXAMPLE: RT-PCR with Oligo d(T)

RT-PCR 1st Strand Synthesis	
Total RNA	50-100ng
Oligo d(T)	0.1 $\mu$ g
Water	Bring up to 10 $\mu$ l
<b>FINAL VOLUME</b>	<b>10 <math>\mu</math>l</b>

► Heat at 65°C for 5 min. Cool to room temperature for 10 min. then add:

10x buffer	2 $\mu$ l
dNTP (10 mM each)	1 $\mu$ l
RNAse inhibitor	20U
RT enzyme	50U

► Heat at 42°C for 60 min, then 70°C for 15 min.

RT-PCR 2nd Strand Synthesis	
cDNA	1 $\mu$ l
10x buffer	5 $\mu$ l
dNTP (10 mM each)	1 $\mu$ l
Primer F (10 $\mu$ M)	1 $\mu$ l
Primer R (10 $\mu$ M)	1 $\mu$ l
DNA Taq Pol	2.5-5U
<b>PCRboost</b>	<b>bring up to 50 <math>\mu</math>l</b>
<b>FINAL VOLUME</b>	<b>50 <math>\mu</math>l</b>

► Proceed with PCR Cycling protocol (refer to table on previous page).