

# Real Time PCR Master Mixes

## GreenDYE Master Mix

## Hot Start QPCR Master Mix

### Basic Protocol for 2x Green DYE Master Mix:

Component	Volume (µl)	Final Conc.
2x Master Mix	25	1x
Primer mixture	15	50-100nM
Template	15	100-300nM

The concentration of primer in the mixture should not exceed 100 nM. Higher concentrations result in unspecific amplification and do not lead to more product. Recommended melting temperature of primers is 53-60C.

### Cycling Program:

Step	Temp (°C)	Duration	Cycles
Enzyme activation	95	10 min	1
Denaturation	95	30 sec	45
Annealing	60	60 sec	
Elongation	72	60 sec	
	95	60 sec	

Store at -20°C. Multiple freeze-thaw-cycles should be avoided by preparing aliquots.

### Basic Protocol for 2x Hot Start QPCR Master Mix

Component	Volume (µl)	Final Conc.
2x Master Mix	25	1x
Primer mixture	15	300 nM
Probes	15	200 nM
Template	15	1-10ng/50µl
Water	fill up to 50µl	

In order to correct possible losses in the sample preparation, it is advised to prepare all reaction solutions in an excess of 5 %. It is recommended to first pipett the primer mixture, then to add the template and last the Master Mix.

We suggest the user reserve plate positions for the use of positive (control DNA) and negative (water or buffer) samples.

Recommended melting temperature of primers is 60C.

Step	Temp (°C)	Duration	Cycles
Enzyme activation	95	10 min	1
Denaturation	95	15 sec	45
Annealing/Extension	60	60 sec	

Store at -20°C. Multiple freeze-thaw-cycles should be avoided by preparing aliquots.