

## Standard DNA amplification assay

### Usage (PCR protocol)

To prevent from unwanted (unspecific) product or primer-dimers formation, it is recommended to set up the pipetting procedure on ice, or to use the Genaxxon HotStart master mixes.

### Add to your PCR tube

The protocol below serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA or primer may vary and must be determined individually.

Pipette the following into a PCR tube, mix and make up to a final volume of 50  $\mu$ L (25  $\mu$ L).

We recommend dispensing all reagents on ice. It is important to vortex all solutions (incl.  $MgCl_2$ ) before use to remove any gradients that may result from repeated freeze/thaw steps.

If you do have already your own PCR-Protocol established, please use your existing pipetting scheme and Thermocycler protocol.

Component	50 $\mu$ L reaction		25 $\mu$ L reaction	
	Volume	Final concentration	Volume	Final concentration
2X <i>RedTaq</i> Mastermix	25 $\mu$ L	1X	12,5 $\mu$ L	1X
Forward Primer	Variable	0.1 – 1 $\mu$ M *	Variable	0.1 – 1 $\mu$ M *
Reverse Primer	Variable	0.1 – 1 $\mu$ M *	Variable	0.1 – 1 $\mu$ M *
Template DNA	Variable	100 pg – 500 ng **	Variable	100 pg – 500 ng **
Sterile deionized water	Up to 50 $\mu$ L	---	Up to 25 $\mu$ L	---

Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube. Overlay the sample with mineral oil or add an appropriate amount of wax if the thermal cycler is not equipped with a heated lid. Place the samples in a thermocycler and start a PCR program.

\* 1  $\mu$ M primer final concentration corresponds with 4-7  $\mu$ L of 3  $\mu$ M stock solution (10-20 pmol absolute).

\*\* 10 pg – 500 ng template DNA mean as a rule of thumb: 0.1 ng – 10 ng of plasmid DNA and 5 ng – 500 ng genomic DNA

**Table 1:**  $MgCl_2$  concentration in a 50  $\mu$ L reaction (*RedTaq* Mastermix)

Final $MgCl_2$ conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25 mM $MgCl_2$ per reaction ( $\mu$ L)	0	1	2	3	4	5	6

### Notes:

- Drops should be collected by centrifugation and 50  $\mu$ L of mineral oil should be layered upon the reaction mixture.
- Program the thermal cycler according to the manufacturer's instructions. Each programme should start with an initial heat incubation step at 94°C for 3-5 minutes!
- **Recommended elongation time is 1 minute per 1kb of target!**
- For maximum yield and specificity, temperatures (annealing) and cycling times should be optimised for each new template target or primer pair.