Quick-Start Protocol October 2017

AllTaq™ Master Mix Kit

The AllTaq Master Mix Kit (cat. nos. 203144, 203146) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. The AllTaq Master Mix can also be stored at 2–8°C for up to 6 months, depending on the expiration date.

Further information

- *AllTaq PCR Handbook*: **www.qiagen.com/HB-2481**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- AllTaq DNA Polymerase requires a heat-activation step of 2 min at 95°C or 3 min at 93°C for long amplicons.
- It is not necessary to keep PCR tubes on ice as nonspecific DNA synthesis cannot occur at room temperature due to the inactive state of AllTaq DNA Polymerase.
- AllTaq PCR Kits are designed to be used with a final primer concentration of 0.25 µM.
- The blue and orange dyes in the Template Tracer and in the Master Mix Tracer, respectively, allow tracking of pipetted samples during the PCR setup. When the blue template is added to the orange Master Mix, the color changes to green. The use of these tracers is optional. Both tracers neither affect sample stability nor PCR performance.

- The blue Template Tracer is provided as a 25x concentrate and should be diluted (using water) to obtain a 1x final concentration in the sample*. To generate a template dilution series, dilute the 25x concentrate (using template and water) to obtain a final concentration of 1x Template Tracer.
- The Master Mix Tracer is provided as a 125x concentrate and can be either added to the reaction setup (Table 1) to obtain a 1x final concentration or it can be added directly to the Master Mix vial† for long-term storage.
- Reactions can be directly loaded onto agarose gels after cycling. Each tracer dye allows monitoring of the loading process and efficient tracking during electrophoresis. The dyes run at about 50 bp (orange) and 4000 bp (blue) on a 1% agarose gel.

1. Thaw AllTaq Master Mix, template DNA or cDNA, primer solutions, RNase-free water, Template Tracer (optional), Master Mix Tracer (optional). Mix thoroughly before use.
2. Prepare a reaction mix according to Table 1. The reaction mix contains all the components except the template DNA. Prepare a volume of reaction mix 10% greater than required for the total number of reactions to be performed.

   It is not necessary to keep samples on ice during reaction setup or while programming the cycler.

   Note: A negative control (without template) should be included in every experiment.
3. Mix the reaction mix gently but thoroughly, for example, by pipetting up and down a few times or vortexing for a few seconds. Dispense appropriate volumes into PCR tubes or wells of a PCR plate.

* Example: add 0.2 µl of the blue Template Tracer (25x) to 5 µl sample before use. If pipetting volumes are too small to handle, the Template Tracer can be pre-diluted using sterile water. In this example, 2 µl of 1:10 pre-diluted Template Tracer could be added.

† Example: add 40 µl of the Master Mix Tracer (125x) to 1 tube (1.25 ml) AllTaq Master Mix (4x). Since the amount of Master Mix tracer added is very small, the concentration of the Master Mix will not be changed and the AllTaq Master Mix can be used as indicated in the protocol

Table 1. Reaction setup for AllTaq Master Mix Kit

Component	Volume/reaction	Final concentration
AllTaq Master Mix, 4x	5 µl	1x
Primer A	Variable	0.25 µM
Primer B	Variable	0.25 µM
RNase-free water	Variable	–
Optional: Master Mix Tracer,125x	0.16 µl	1x
Template DNA (added at step 4)	Variable	0.1 pg – 1 µg/reaction
Total reaction volume	20 µl	

4. Add template DNA (1 µg – 100 fg per reaction, depending on target abundance) to the individual PCR tubes. The AllTaq Master Mix Kit can be used with genomic DNA, cDNA, plasmid DNA, oligonucleotides and other DNA molecules as template.

   Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 2 and 3.

5. Place the PCR tubes or plates in the thermal cycler and start the PCR program.

   Note: After amplification, samples can be stored at –20°C for longer storage.

6. We have evaluated several specialized protocols and particular hints. For details, please refer to the *AllTaq PCR Handbook*.

Table 2. AllTaq cycling conditions for amplicons ≤ 1 kbp

Step	Time	Temperature	Comment
Initial PCR activation	2 min	95°C	This heating step activates AllTaq DNA Polymerase.
3-step cycling:			
Denaturation	5 s	95°C	Do not exceed this temperature.
Annealing	15 s	55°C	Approximately 5°C below T_m of primers.
Extension	10 s	72°C	For PCR products up to 1000 bp, an extension time of 10 s is sufficient.
Number of cycles	40		The optimal cycle number depends on the amount of template and the abundance of the target.

Table 3. AllTaq cycling conditions for amplicons 1–9* kbp

Step	Time	Temperature	Comment
Initial PCR activation	3 min	93°C	This heating step activates AllTaq DNA Polymerase.
3-step cycling:			
Denaturation	15 s	93°C	Do not exceed this temperature.
Annealing	30 s	60°C	Approximately identical to T_m of primers.
Extension	1 min/kb	68°C	Allow 1min per kbp amplicon size.
Number of cycles	40		The optimal cycle number depends on the amount of template and the abundance of the target.

* Performance in amplification of long targets depends on the quality of the template and assay.

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