Quick-Start Protocol October 2017

AllTaq™ PCR Core Kit

The AllTaq PCR Core Kit (cat. nos. 203123, 203125 and 203127) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer.

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 Buffer 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.
- The AllTaq PCR Core Kit is provided with Q-Solution® which facilitates amplification of templates that have a high degree of secondary structure or that are GC-rich. When using Q-Solution for the first time with a particular primer–template system, always perform parallel reactions with and without Q-Solution.
- AllTaq PCR buffer contains Mg^{2+}. If addition of more Mg^{2+} is desired, the optimal concentration should be determined empirically by adding provided $MgCl_2$ solution in steps of 0.5 mM, corresponding to 0.5 µl in a 25 µl reaction.

1. Thaw AllTaq PCR Buffer, dNTP-Mix, template DNA or cDNA, primer solutions, RNase-free water, $MgCl_2$ (optional), Template Tracer (optional), Master Mix Tracer (optional) and 5x Q-Solution (optional). Mix thoroughly before use.

* 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 48 µl of the Master Mix Tracer (125x) to 1 tube (1.2 ml) AllTaq PCR Buffer (5x). Since the amount of Master Mix Tracer added is very small, the concentration of the buffer will not be changed and the AllTaq PCR Buffer can be used as indicated in the protocol.

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.

Table 1. Reaction setup for AllTaq PCR Core Kit

Component	Volume/reaction	Final concentration
AllTaq PCR Buffer, 5x	5 µl	1x
AllTaq DNA Polymerase	0.5 µl	2.5 U/rxn
dNTP-Mix, 10 mM each	0.5 µl	0.2 mM
Primer A	Variable	0.25 µM
Primer B	Variable	0.25 µM
RNase-free water	Variable	–
Optional: $MgCl_2$, 25mM	Variable*	–
Optional: Master Mix Tracer,125x	0.2 µl	1x
Optional: 5x Q-Solution†	5 µl	1x
Template DNA (added at step 4)	Variable	0.1 pg – 1 µg/reaction
Total reaction volume	25 µl	

* If needed, determine optimal concentration by sequentially adding 0.5 µl, 1 µl, 1.5 µl, etc., of provided $MgCl_2$ solution.

† For templates with GC-rich regions or complex secondary structure.

4. Add template DNA (1 µg – 100 fg per reaction, depending on target abundance) to the individual PCR tubes. The AllTaq PCR Core 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 the 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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