DB-1277 DB AptaTaq DNA Polymerase

General description

DB AptaTaq DNA Polymerase is a hot-start version of the *Taq* DNA Polymerase with an aptamer inhibitor. The aptamer allows the PCR reaction to be prepared at ambient temperature without cooling the mixture, as the polymerase activity is completely inhibited at the temperature below 40 °C. Under normal cycling conditions, the aptamer is released, and polymerase activity is not influenced at temperature or above 55 °C. This inhibition is reversible, and the annealing temperature should thus be at 55 °C or higher.

DB AptaTaq DNA polymerase is recombinantly expressed (in *E. coli*) highly thermostable DNA polymerase from the thermophilic bacterium *Thermus aquaticus*. The enzyme catalyzes primer-initiated synthesis of the complementary DNA strand in the 5' → 3' direction and has 5' → 3' exonuclease activity, which is required for quantitative PCR with hydrolysis probes (e.g. TaqMan™ probes). *Taq* DNA Polymerase does not have 3' → 5' exonuclease (proofreading) activity, but exhibits deoxynucleotidyl transferase activity, which results in the addition of extra adenine (A) at the 3'-end of the PCR product. This extra adenine on the PCR products can be used for T/A-cloning.

DB AptaTaq DNA Polymerase is supplied with a complete reaction buffer, PCR Buffer A (5X), which contains all necessary components including enhancers, stabilizers, and optimal concentration of KCl, $MgCl_2$ and dNTPs. No other components need to be added to run a standard PCR reaction.

Kit Components

Kit component	REF code	Volume (µL)			Storage temperature	Cap colour + label
		0.1 kU	0.5 kU	2.5 kU		
AptaTaq DNA Polymerase	RF06710	20	100	5 x 100	-18 °C to -25 °C	Apta Taq
PCR Buffer A (5X)	RF07989	1x 1 000	2 x 1 000	10 x 1 000	≤ -18 °C	5x

Storage

Keep AptaTaq DNA Polymerase at -18 °C to -25 °C for long-term storage. We recommend using cooling block while working with the Polymerase.

PCR buffer A (5X) should be stored at ≤ -18 °C for long-term storage. Avoid more than 5 freeze/thaw cycles. If you intend to use the components more times, aliquot them after the first thawing.

PCR preparation

Prepare PCR reagents.

- Thaw PCR Buffer A (5X) and mix well. Collect the liquid by centrifugation of the vial before opening.

Prepare the Reaction Mix and add the DNA template.

- It is recommended to prepare the components with a 10 % excess. It is advantageous to prepare "master mixes" of several components for multiple reactions whenever possible.
- The recommended reaction volume in standard PCR tubes or 8 or 12-well strips or 96-well plate is 20 µL: the volume of PCR Buffer A (5X) per reaction is 4 µL, the volume of other components depends on their actual concentration – PCR Buffer A (5X), AptaTaq DNA Polymerase, primers, probe(s), template(s) and water should result in a 20 µL reaction.
- Add AptaTaq DNA Polymerase to the mixture of PCR Buffer (5X), primers, probe(s) and water. Mix well after addition of the polymerase or at least when you are dividing the master mix into different wells – polymerase contains glycerol.
- Add the sample (DNA template) as the last component. DB AptaTaq DNA Polymerase can also be used for direct detection (e.g. in colony PCR) but each application must be validated by the user, and we recommend using our PCR mixes for direct detection. For direct PCR, the volume of the sample is usually limited as a higher amount of the sample may inhibit PCR reaction.
- Close the PCR tubes or strips (or seal the qPCR plate with a foil) and centrifuge briefly to collect the liquid at the bottom of the wells. For quantitative PCR with real-time fluorescence detection, use appropriate tubes or strips or plates and foils (clear, frosted, or white wells depending on your instrument, optically transparent caps, or foils).
- See Table 1 for the summary of volume and concentration of each component of the reaction.

Table 1: Composition of the reaction

Component	Final concentration or amount	Volume per 20 µL reaction	Volume per 10 µL reaction[1)]
PCR Buffer A (5X)	1X	4 µL	2 µL
AptaTaq DNA Polymerase[2)]	0.05-0.1 U/µL[3)]	0.2-0.4 µL	0.1-0.2 µL
Forward primer(s)	250-1000 nM (each) [4)]	Variable	Variable
Reverse primer(s)	250-1000 nM (each) [4)]	Variable	Variable
Probe(s)	100-250 nM (each) [5)]	Variable	Variable
DNA template/ biological sample, cell culture [6)]	2 pg – 100 ng/ 10-20 %	2-10 µL/ 2-4 µL	2-5 µL/ 1-2 µL
PCR grade water		Up to 20 µL	Up to 10 µL

[1)] Recommended volume for use with 384-well qPCR plate.
[2)] Stock concentration – 5 U/µL.
[3)] 0.05 U/µL is recommended for most applications. Higher concentration may be required for some biological samples and matrices.
[4)] 400 nM concentration is recommended if you do not want to optimize the concentration.
[5)] 100 nM concentration is recommended if you do not want to optimize the concentration.
[6)] For direct PCR detection, a sample volume of 2 µL per 20 µL reaction is recommended for most samples, sample volume of more than 4 µL will likely inhibit the PCR reaction. A quantity of 1 000 cells is recommended for the first test to see if the organism to be tested is suitable for direct PCR. Each direct PCR application must be validated by the user.

PCR protocol

Step	Cycles	Temperature	Time (min:sec)
Initial denaturation	1	94 °C	2:00 [1)]
Amplification - Denaturation - Annealing - Extension	45	 94 °C 60 °C [2)] 72 °C	 00:05 00:15 00:15 [3)]
Cooling/Hold if needed	1	4 °C	00:30 up to 24 h

[1)] Increase the time of the initial denaturation time to up to 10 minutes for direct PCR detection (such as colony PCR) in case you have poor sensitivity. Please note, that some media can precipitate at such temperature.

[2)] Adjust the annealing temperature according to the primers used. The annealing temperature should not be below 55 °C due to possible inhibition of the polymerase by the aptamer.

[3)] In the case of amplification of amplicons longer than 250 bases, increase the extension time to at least 1 minute per 1 kilobase.

Run PCR

Place the tubes or strips or plate in the PCR instrument. In the case of real-time PCR, select detection in the appropriate channels based on the probe(s). Start the cycling program.

Disclaimer

For research use only.

It is the user´s responsibility to validate the specific use of the kit.

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Troubleshooting Guide

Poor sensitivity: little or no amplification (applies for both end-point and real-time PCR)	
a) Insufficient amount of template	The amount of template may be too low. • Quantify the concentration using OD_{260} or any fluorescence-based method to ensure using the amount of template as recommended. • Try to add more sample to the reaction, ideally also use internal positive control to see if the reaction is inhibited.
b) PCR inhibitors	Samples of purified DNA can contain inhibitors from the sample or compounds used during nucleic acid extraction which inhibit PCR. • Perform control measurement of ratios A_{260}/A_{230} and A_{260}/A_{280} to evaluate the purity of DNA. • Add extra steps to the nucleic acid extraction to gain DNA of higher purity or use lower volume of the DNA isolate. • Use higher concentration of AptaTaq DNA Polymerase or increase extension time. Or add less of the sample to the reaction. • Use an internal positive control to see the extent of PCR inhibition.
c) Biological matrices/ cultivation media	Biological matrices such as saliva or media can inhibit PCR. • Pre-heating (specific for individual matrix/medium) before adding to the final reaction can help to increase the sensitivity of PCR. E.g. incubating media at 65 °C for 15 minutes or 80 °C for 5 minutes, or incubating saliva at 80 °C for 10 minutes. • For colony PCR, extension of the initial denaturation to 10 min at 94 °C is recommended.
d) Template degradation	DNA template may be degraded. • Prepare a new sample or a use a different aliquot if available.
e) Primer-dimers	Primers may form primer-dimers and lower the sensitivity. • Try to change the primer concentration. • Try to optimize the annealing temperature. • Redesign primers or try our ultrasensitive PCR mix DB-1273.
f) Mispriming	Primers may anneal to the incorrect location on template. • Consider a new primer design. • Increase the annealing temperature.
g) Incorrect annealing temperature	Annealing temperature can be too high. • Optimize the annealing temperature for primers.
h) Short extension time	• Use longer extension time for longer amplicons. It is recommended to set the extension time to 1 min/1 kb.
Low fluorescence intensity (applies only for real-time PCR with fluorescent probes)	
a) Primer-dimers	Primers may form primer-dimers and lower the sensitivity. • Try to change the primer concentration. • Try to optimize the annealing temperature. • Redesign primers or try our ultrasensitive PCR mix DB-1273.
b) Low probe concentration	• Increase the probe concentration. It is recommended to use each probe at concentration 100-250 nM.
c) Wrong detection channel	• Ensure the correct detection channel(s) is used in relation to the probe(s)/ SYBR™ Green I dye used.

Positive signal in no template control (applies for real-time PCR with fluorescent probes or SYBR™ Green)	
a) Contamination	• Discard all contaminated solutions. Prepare the reagents in a clean PCR box to avoid contamination of kit components. Use filter tips. • Add the sample at a separate location from where you prepare the PCR master mix. Do not handle samples or PCR products where you prepare the master mixes, keep them separate from PCR reagents.
Positive signal in no template control (applies only for real-time PCR with SYBR™ Green)	
a) Primer-dimers with SYBR™ Green I dye	Primers may form primer-dimers. • Try to change primer concentration. • Optimize the annealing temperature. • Consider designing a gene-specific hydrolysis probe (e.g. TaqMan™) without further use of SYBR™ Green I dye. • Redesign primers or try our ultrasensitive PCR mix DB-1274.
Significant differences in results among replicates	
a) Reagents or reaction mixes not properly mixed	• Mix reagents thoroughly after thawing. Ensure the reaction mixes are mixed well.
b) Sample evaporation	• Make sure the PCR plate is properly sealed to avoid evaporation.
c) Bubbles in reaction wells	• Centrifuge the PCR plate before running PCR to remove bubbles.