AptaHotTaq DNA Polymerase

Description: *AptaHotTaq* DNA Polymerase is a HotStart DNA Polymerase using DNA-Aptamers instead of antibodies for blocking the activity at ambient temperature. At temperatures above 50°C the DNA-aptameres reversibly dissociate from the enzyme. The polymerase is activated during normal cycling conditions, allowing for a convenient assembly of PCR reactions at room-temperature.

Content

Ref No.	119630HC	color
AptaHotTaq DNA Polymerase	1000 units	blue
Incomplete NH_4 * Reaction Buffer (10x)	2x 1.8 mL	red
Complete NH_4 ** Reaction Buffer (10x)	2x 1.8 mL	yellow
Complete KCl *** Reaction Buffer (10x)	2x 1.8 mL	black
$MgCl_2$ 100 mM	1 mL	green
Datasheet	1	--

* Incomplete NH_4 Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, free of $MgCl_2$.

** Complete NH_4 Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, 20 mM $MgCl_2$.

*** Complete KCl Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, 15 mM $MgCl_2$.

Applications: *AptaHotTaq* DNA Polymerase is suitable for all regular applications but especially for PCR with complex genomic or cDNA templates, low copy number targets, large number of thermal cycles, Multiplex and Real Time PCR. *AptaHotTaq* Polymerase effectively directs PCR with templates up to 5 kb in length. Suitable for diagnostic kits in lyophilised formats.

Concentration: 30 Units/µL

Sensitivity: detection of ≥ 6 DNA molecules

Unit definition: One unit of activity is defined as the amount of enzyme required to incorporate 10 nmoles of dNTP into acid-insoluble DNA fraction in 30 minutes at 72 °C.

Recommended $MgCl_2$ concentration: 1.5 mM – 6 mM

Quality Control

- 98% protein homogeneity in 10% SDS-PAGE
- No detectable exo-/endonuclease activities
- PCR amplification tests with different templates
- PCR amplification tests without templates as Negative Control
- Hotstart efficiency test showing effective blockage by specific aptamers
- Exonuclease efficiency test showing efficient 5´ - 3´Exonuclease activity

Storage condition: -20 °C

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Pipetting scheme and thermocycler protocol

Components	Volume / 50 µL Reaction	Final concentration
10 x PCR-Buffer	5 µL	1 x
dNTP-Mix (10 mM each)	1 µL	200 µM each
Upstream Primer	variable	0.1 - 0.5 µM
Downstream Primer	variable	0.1 - 0.5 µM
AH-Taq DNA Polymerase 5 U/µL	0.25 - 1.0 µL	1.25 - 5.0 units
Template DNA	variable	10 to 500 ng/reaction
Sterile dest. water	Adjust to 50 µL final volume	

Separate $MgCl_2$ solution can be used for optimization. If incomplete buffer is used, **titrate $MgCl_2$** for optimal PCR results with following recommendation (see table):

Final $MgCl_2$ conc. [mM]	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
Volume [µL] of 100 mM $MgCl_2$/ 50 µL	0.75	1	1.25	1.5	1.75	2	2.25	2.5	2.75	3

Thermocycler protocol

step	time	temperature
initial denaturation	2 minutes	94 °C
30 cycles:		
denaturation	10 seconds	94 °C
annealing	20 seconds	55 - 68 °C *
extension	1 minute	72 °C

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers.

Notes:
Program the cycler according to the manufacturer's instructions.
Each program should start with an initial denaturation step at 94 °C for 2 to max. 5 min.
Recommended elongation time: 1 min/ 1 kb of target.
For maximum yield and specificity, temperatures (annealing) and cycling times should be optimized for each new template target or primer pair.

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