GENETEK
BIOPHARMA

GT HSTaq™ DNA Polymerase
Genetek Hot Start Taq DNA Pol

CAT# GT-21201

Product Description

Each pack of GT HSTaq DNA Pol enzyme contains 10X Buffer and MgCl2.

Genetek Biopharma's HSTaq™ DNA Polymerase is a heat stable Taq DNA polymerase in a proprietary formulation. It is a highly processive 5'→3' DNA polymerase, lacking 3'→5' exonuclease or proofreading activity. This recombinant enzyme reaches its peak enzymatic activity at 72°C.

The enzyme is heat stable therefore it can withstand high temperature for much longer time than GTTaq DNA pol. Its activation requires preheating for 5-20 min depending on the type of PCR. If you use it in kits other than Genetek kits, please try to optimize preheating time needed. Preheating is usually carried out at 95 ℃ for about 15-20 min.

To improve overall enzymatic activity of the enzyme, Mg^{+2} is needed. Therefore, MgCl2 is provided as a separate solution to be added to the 10X Buffer upon use. Usually 1.5 mM is used. MgCl2 has the ability to increase the rate of non-specific binding during the amplification process, so its optimal concentration is important.

For research use only. Not for diagnosis or clinical application.

GT HS Taq™ DNA Polymerase PCR Protocol

- Use 1 µl (5 units) of the GTHS Taq™ DNA Polymerase for each 25 µl PCR preparation.
- Due to multiple factors affecting amplification process, you may adjust the amount of GTHS Taq DNA pol required for an ideal condition, however, we recommend using 1 ul or 5 units of enzyme per 25 ul PCR.

Recommended Protocol and Conditions for GTHSTaq™ DNA Polymerase in a PCR

	PCR Components		Volume	Final concentration
1	dNTP 40 Mm (10 mM each)		1 µl	800 µM (200 µM each)
2	Primer (10 pmol stock)	Forward	0.5 to 2.5 µl each	0.1 – 0.5 µM
		Reverse		
3	10X Buffer		5 µl	1X
4	$MgCl_2$ (100 mM)		0.75 to 1 µl	1.5 – 2 mM
5	Template DNA		10 pg - 1 µg	-
6	KBC HS Taq™ DNA Polymerase (5U/µl)		0.5 µl	2.5 Unit
7	Sterile distilled water		Up to 50 µl	-

Most primer manufacturers recommend that the primers be diluted in a given amount of dH2O. If the lyophilized oligo is diluted in the given volume, then one would have 100 pmol/µl. From this a 10 pmol working solution is made by diluting in dH2O, and 5-10 pmol/PCR from each primer is used.

Initial step	Cycling			Final Extension	Storing in Cycler
	Denaturation	Annealing	Extension		
95 ˚C	95 ˚C	63 ˚C or any temp suitable for your primers	70 ˚C	70 ˚C	4 ˚C
20 min	1 min	90 sec	2 min	17-20 min	∞
	27-30 Cycles				

1.Due to the sensitivity of the long range PCR amplification process to minute changes in the environment, devices utilized for the amplification process, and other overall conditions, the values in these tables may not remain constant and are also susceptible to modification by the user. In such cases, follow the recommended PCR protocol and guidelines prior to altering the PCR program or any of the reaction conditions to gauge the initial deviation. You may change the device setup and other conditions following the evaluation.

2.The optimal annealing temperature depends on the primer melting temperature and the devices used for the PCR process. Hot start

3.The duration of the extension step depends on the of the length of the DNA fragment to be amplified.

Troubleshooting

No.	Problem	Potential Cause	Potential Solution
1	No PCR product or low yield	Poor template DNA quality	Check DNA quality and concentration
			Analyse DNA using gel electrophoresis
			Use control DNA simultaneously during the PCR process
			Check DNA purity
		Low amount of enzyme	Slightly increase the amount of enzyme
		Enzyme exposed to higher temperatures	Add the enzyme during the last regent preparation step before thermal cycling as to avoid its heat inactivation or use GT HS Taq DNA pol. This enzyme is more heat stable
		Low $MgCl_2$ concentration	increase $MgCl_2$ concentration
		Poor quality dNTP Mix	Use higher quality dNTP mix. The optimal concentration range for PCR amplification is between 100 and 250 µM.
		Number of PCR cycles is insufficient	Increase annealing temperature
			Increase the number of PCR cycles
			Ensure that the final extension step was completed
		Suboptimal Primer design	Review and redesign the primers
		suboptimal primer concentration	Ensure that both primers are at the required default equal concentration
			Check primer concentration; Vary concentration to achieve optimal primer concentration if necessary.
		Primers stored under suboptimal conditions	Use control DNA simultaneously during the PCR process to ensure that the primers are not degraded
			Store primers between -25 and -15 °C.
2	Multiple additional/non-specific bands or excessive smearing on the gel	Low annealing temperature	Increase annealing temperature
		Long extension period	Lower duration of the extension step
		suboptimal primer concentration and design	Ensure that both primers are at the required equal concentration
			Ensure that both primers have an equal melting temperature
		Suboptimal DNA concentration	Adjust DNA concentration and analyse the product to determine optimal DNA concentration
		High enzyme concentration	Decrease enzyme amount or concentration

For any further information, please contact our technical service via email: (support@genetek.de).