HotStart Taq DNA Polymerase basic kit, 500 U
(Cat No: PD012S)

Description:
Hot Start Taq DNA polymerase is a thermostable DNA polymerase manufactured with the highest standards. It is certified as RNase and DNAse free, that guarantee reproducible and efficient PCR reactions.
Taq DNA Polymerase is a thermostable enzyme that catalyzes 5'→3' synthesis of DNA. The enzyme has no detectable 3'→5' proofreading exonuclease activity but possesses low 5'→3' exonuclease activity. Hot Start Taq DNA Polymerase has high processivity up to 5 kb, while it can reliably amplify a substrate as low as 6-12 copies per reaction.

Source:
The Enzyme is purified from an *E. coli* strain carrying a plasmid with Taq DNA polymerase gene from *Thermus aquaticus* YT-1. After production, it is chemically modified into a Hot Start enzyme, that requires excessive thermal activation while ensuring high specificity.

Unit definition:
One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmoles of dNTPs into acid insoluble material in 30 minutes at 72°C.

Reagents supplied:

PD012S kit		
Cat No	**Description**	**Tubes No.**
PD002XS	HotStart Taq DNA polymerase, 5u/µl, 500 U (50 mM Tris-HCl (pH 7.9 @ 25°C), 50 mM KCl, 0.1 mM EDTA, 50% glycerol, 0.5% IGEPAL, 1 mM DTT, 0.5% Tween-20)	1
BR006-1.4	10x Taq DNA polymerase Buffer with 15mM $MgCl_2$, 1.4 ml (100 mM Tris-HCl (pH 8.3), 500 mM KCl, 15mM $MgCl_2$)	1
BR007-1.4	10x Taq DNA polymerase Buffer w/o $MgCl_2$, 1.4 ml (100 mM Tris-HCl (pH 8.3), 500 mM KCl)	1
BR008-1.4	25mM $MgCl_2$, 1.4 ml	1
BR028-250	dNTPs mix 10mM each, 0.250 ml	1
BR033-1.8	Nuclease Free Water, 1.8 ml	2

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PCR Guidelines:

Reactions may be set at room temperature.

PCR recommended reaction:

Components	**25µl assay**	**Final Concentration/ Quantity (25µl assay)**	**50µl assay**	**Final Concentration/ Quantity (50µl assay)**
10x Taq pol. Buffer with 15mM $MgCl_2$	2.5µl	1x	5µl	1x
dNTP mix 10mM each	0.5µl	0.2 mM each	1µl	0.2 mM each
10µM forward primer	0.5µl	0.2-0.8 µM	1 µl	0.2-0.5 µM
10µM reverse primer	0.5µl	0.2-0.8 µM	1 µl	0.2-0.5 µM
Template DNA	Variable	10 fg-500 ng*	Variable	100 fg-500 ng*
Hot Start Taq DNA pol. (5u/µl)	0.25µl	1.25 u/25 µl reaction (0.05 u/µl)	0.25-0.5µl	1.25-2.5 u/50 µl reaction (0.025-0.05 u/µl)
Sterile ultrapure water	Up to 25 µl		Up to 50 µl	

*** Substrate quantity is mostly dependent on the complexity and the purity of substrate and should be defined after testing for each primer set/substrate combination. For highly pure DNA substrates (OD260/OD280 ~ 2 and OD260/OD230~ 2,2) dissolved in water for molecular biology, 5-10 copies of specific target substrate are enough for end product gel visualization.**

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PCR recommended conditions:

Step	Temperature	Time
Initial denaturation/Activation	**95°C**	**15 min**
25-40 cycles		
Denaturation	95°C	30sec
Annealing	45-68°C*	20sec
Extension	72°C	1min/kb
Final extension	72°C	5 min
Hold	4-12°C	Indefinitely

**Annealing temperature depends on primers' Tm*

Initial activation for 15 minutes is very important in the case of reaction with very low PCR template copies and for obtaining the maximum efficiency in qPCR setups (either Taqman or Sybr Green chemistry). DO NOT USE LOWER THAN 10 min!!

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For qPCR or one step RT-qPCR applications

Hot Start Taq DNA polymerase is compatible with qPCR applications.

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DNA qPCR

PCR recommended reaction:

Taq Man qPCR with kit components			**Taq man qPCR EQ protocol**		
Components	**Standard 20µl assay**	**Final Concentration/ Quantity (20 µl assay)**	**Components**	**Standard 20µl assay**	**Final Concentration/ Quantity (20 µl assay)**
10x Taq pol. Buffer w/o $MgCl_2$	2µl	1x	**5x One Step RT-qPCR buffer*** (BR030)	5 µl	1x
25mM MgCl2	2 µl	1.5 -4 mM			
dNTP mix 10mM each	0.4µl	0.2 mM each			
10µM forward primer	0.8µl	0.2-0.8 µM For single target 0.4 µM for multiplex 0.2 µM or lower	10µM forward primer	0.8 µl	0.2-0.8 µM For single target 0.4 µM for multiplex 0.2 µM or lower
10µM reverse primer	0.8µl	0.2-0.8 µM For single target 0.4 µM for multiplex 0.2 µM or lower	10µM reverse primer	0.8 µl	0.2-0.8 µM For single target 0.4 µM for multiplex 0.2 µM or lower
10 µM Taqman Probe	0.4 µl	0.2-0.5 µM (~ half or lower than the primer conc.)	10 µM Taqman Probe	0.4 µl	0.2-0.5 µM (~ half or lower than the primer conc.)
Template DNA	Variable	10 fg-500 ng	Template DNA	variable	10 fg-500 ng

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Hot Start Taq DNA pol. (5u/μl)	0.25-0.5 μl	1.25-2.5 u/20 μl reaction (0.0625-0.125 u/μl)	Hot Start Taq DNA pol. (5u/μl)	0.25-0.5 μl	1.25-2.5 u/20 μl reaction (0.0625-0.125 u/μl)
Sterile ultrapure water	Up to 20 μl	N/A	Sterile ultrapure water	Up to 20 μl	N/A

***BR030 may be separately purchased. It contains an EQ optimized chemistry for qPCR applications along with dNTPs.**

Similar setup is followed for Sybr Green based qPCR assays. Instead of Probe one may add Sybr Green in final concentrations of 0.2-2x.

Recommended conditions:

Step		**Temperature**			**Time**
Initial denaturation/Activation		**95°C**			**15 min**
3 steps program			**2 Steps program**		
Denaturation	95°C	10-15 sec	Denaturation	95°C	10-15 sec
Annealing	45-68°C*	10-20 sec	Annealing/ extension	(Tm-1°C)*	30 sec
Extension	72°C	1min/kb (6 sec for 100 bp ampliqons)			

**Annealing temperature depends on primers' Tm. For two steps programs the primers are designed to have a Tm ~ 68. For most primers sets tested, optimal Ta was found at 1-2°C below the lower Tm of the primer set.*

Initial activation for 15 minutes is very important in the case of reaction with very low PCR template copies and for obtaining the maximum efficiency in qPCR setups (either TaqMan or SYBR Green chemistry). DO NOT USE LOWER THAN 10 min!!

One-Step RT qPCR

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PCR recommended reaction:

One Step RT-qPCR with kit components			Taq man qPCR EQ protocol		
Components	**Standard 20µl assay**	**Final Concentration/ Quantity (20 µl assay)**	**Components**	**Standard 20µl assay**	**Final Concentration/ Quantity (20 µl assay)**
10x Taq pol. Buffer w/o $MgCl_2$	2µl	1x	**5x One Step RT-qPCR buffer*** (BR030)	5 µl	1x
25mM MgCl2	2 µl	1.5 -4 mM			
dNTP mix 10mM each	0.4µl	0.2 mM each			
10µM forward primer	0.8µl	0.2-0.8 µM For single target 0.4 µM for multiplex 0.2 µM or lower	10µM forward primer	0.8 µl	0.2-0.8 µM For single target 0.4 µM for multiplex 0.2 µM or lower
10µM reverse primer	0.8µl	0.2-0.8 µM For single target 0.4 µM for multiplex 0.2 µM or lower	10µM reverse primer	0.8 µl	0.2-0.8 µM For single target 0.4 µM for multiplex 0.2 µM or lower
10 µM Taqman Probe	0.4 µl	0.2-0.5 µM (~ half or lower than the primer conc.)	10 µM Taqman Probe	0.4 µl	0.2-0.5 µM (~ half or lower than the primer conc.)
Template DNA	Variable	10 fg-500 ng	Template DNA	variable	10 fg-500 ng

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Hot Start Taq DNA pol. (5u/µl)	0.25-0.5 µl	1.25-2.5 u/20 µl reaction (0.0625-0.125 u/µl)	Hot Start Taq DNA pol. (5u/µl)	0.25-0.5 µl	1.25-2.5 u/20 µl reaction (0.0625-0.125 u/µl)
Engineered MMLV Reverse transcriptase, 200u/µl (RN001S)	0.05 µl	8-10 u/20 µL reaction (0.4-0.5 u/µl)	Engineered MMLV Reverse transcriptase, 200u/µl (RN001S)	0.05 µl	8-10 u/20 µL reaction (0.4-0.5 u/µl)
Sterile ultrapure water	Up to 20 µl	N/A	Sterile ultrapure water	Up to 20 µl	N/A

***BR030 may be separately purchased. It contains an EQ optimized chemistry for qPCR applications along with dNTPs. RN001S is part of the RN012S kit, which may be separately purchased.**

Similar setup is followed for SYBR Green based qPCR assays. Instead of Probe one may add SYBR Green in final concentrations of 0.2-2x.

One Step-RT recommended conditions:

Step	**Temperature**	**Time**
Reverse Transcription step	55 °C	15 min
Initial denaturation/Activation of Taq DNA polymerase/Reverse Transcriptase deactivation	**95°C**	**15 min**

3 steps program (30-40 cycles)			**2 Steps program (30-40 cycles)**		
Denaturation	95°C	10-15 sec	Denaturation	95°C	10-15 sec
Annealing	45-68°C*	10-20 sec	Annealing/ extension	(Tm-1°C)*	30 sec

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Extension	72°C	1min/kb (6 sec for 100 bp ampliqons)			

**Annealing temperature depends on primers' Tm. For two steps programs the primers are designed to have a Tm ~ 68. For most primers sets tested, optimal Ta was found at 1-2°C below the lower Tm of the primer set.*

For ready to use 2x SybrGreen qPCR mix with Hot Start properties check RN014S kit (EQ 2x qPCR Master Mix Green kit, w/o ROX (TM))

Functional Quality Control:
HotStart Taq DNA Polymerase is tested for performance in the polymerase chain reaction (PCR) using 1.25 units of enzyme to amplify:

a) a 5000-bp region of Lambda DNA using 20ng as substrate in a 50µl reaction. The resulting PCR product is visualized as a single band on agarose gel.

Other Quality Controls:
Certified as DNase-RNase free using fluorescently labeled RNase and DNase probes. More details concerning quality controls could be found in respective Certificate of analysis.

Shipping
Shipped on blue ice or dry ice.

Storage conditions
Store at -20°C ± 5°C

Shelf life:
24 months upon production