Blue TEMPase Hot Start Master Mix C

Based on Combination Buffer

2x Master Mix Kit (1.5 mM $MgCl_2$)

7 312056 230618

Cat. No.: 733-1841 (2500 Reactions)

Cat.No.	No. of 50 µl Reactions	Blue TEMPase Hot Start 2x Master Mix C, based on Combination Buffer
ID No. Cap colour	-	**5200700 Blue**
733-2414	100	2 x 1.25 ml
733-2290	500	10 x 1.25 ml
733-1841	2500	50 x 1.25 ml
733-1842 Sample	20	1 x 0.5 ml

Store at -20 °C.

General Description

Blue TEMPase Hot Start Master Mix C is a ready-to-use 2x master mix. Simply add primers, template, and water to successfully carry out primer extensions and other molecular biology applications.

TEMPase Hot Start DNA Polymerase, the balanced K/NH_4^+ buffer system, dNTPs and magnesium chloride are present in TEMPase Hot Start Master Mix based on Combination Buffer. Each reaction requires 25 µl of the 2x reaction mix. Simply add primers, template and water to a total reaction volume of 50 µl.

TEMPase Hot Start DNA Polymerase is a modified form of VWR Taq DNA Polymerase, which is activated by heat treatment. A chemical moiety is attached to the enzyme at the active site, which renders the enzyme inactive at room temperature. Thus, during setup and the first ramp of thermal cycling, the enzyme is not active and misprimed primers are not extended. The result is higher specificity and greater yields when compared to standard DNA polymerases.

Blue TEMPase Hot Start Master Mix offers several advantages. Set up time is significantly reduced. The chance of contaminating component stocks is eliminated. Reduction of reagent handling steps leads to better reproducibility. Standard tests can be set up with the confidence that results will be consistent every time.

There is no need to use separate loading buffer. Simply load a portion of the reaction product onto an agarose gel for electrophoresis and subsequent visualization. The blue dye front runs at 400 – 500 bp on a 0.5 – 1.5% agarose gel.

Composition of Blue TEMPase Hot Start 2x Master Mix C

- Tris-HCl, pH 8.7, Balanced $KCl/(NH_4)_2S0_4$, 3 mM $MgCl_2$, 0.2 % Tween® 20.
- 0.4 mM dNTPs (each)
- TEMPase Hot Start DNA Polymerase
- Inert blue dye
- Stabilizer

Protocol

This protocol serves as a guideline for PCR. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

Notes:

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis.
- Table 1 shows the reaction set up for a final volume of 50 µl.
- After primer extension, a sample (10% - 30 % of the reaction) can be loaded directly on a gel for analysis.

1. Thaw 2x Master Mix and primers. **It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts.**

   Important: Spin TEMPase Master Mix vials briefly before use.

2. Set up each reaction as shown in table 1.

Table 1. Reaction components: Master mix and template DNA

Component	Vol./reaction*	Final concentration*
TEMPase Hot Start 2x Master Mix	25 µl	1x
25 mM $MgCl_2$**	X µl	1.5 mM (0.5 – 5 mM)
Primer A (10 µM)	1 µl (0.5 – 5 µl)	0.2 µM (0.1 – 1.0 µM)
Primer B (10 µM)	1 µl (0.5 – 5 µl)	0.2 µM (0.1 – 1.0 µM)
PCR-grade H_2O	X µl	-
Template DNA	X µl	genomic DNA: 50 ng (10 – 500 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
TOTAL volume	50 µl	-

* Suggested starting conditions; theoretically used conditions in brackets
** Not included in the kit

3. Mix gently by pipetting the solution up and down a few times.
4. Program the thermal cycler according to the manufacturer´s instructions.

Version: VWR 10/2019

Made in Denmark

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For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

5. Place the tubes in the thermal cycler and start the reaction.

6. At the end of the run, simply load a portion of the reaction product (e.g. 10 µl) onto an agarose gel for analysis.

Three-step PCR Program

Cycles	Duration of cycle	Temperature
1	15 minutes[a]	95 °C
25-35	20 – 30 seconds[b] 20 – 40 seconds[c] 30 seconds[d]	95 °C 50 – 65 °C 72 °C
1	5 minutes[e]	72 °C

[a] For activation of the TEMPase Hot Start DNA Polymerase.

[b] Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 20 – 30 seconds. It causes melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

[c] Annealing step: The reaction temperature is lowered to 50 – 65 °C for 20 – 40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically, the annealing temperature is about 3 – 5 °C below the T_m of the primers used.

[d] Extension/elongation step: TEMPase Hot Start DNA Polymerase has its optimal activity at 72 °C. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand. The extension time depends on the length of the DNA fragment to be amplified. As a rule of thumb, at its optimum temperature the DNA polymerase will polymerize a thousand bases per minute.

[e] Final elongation: This single step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

Related Products

Description	Cat. No.
VWR Taq DNA Polymerase	
VWR Glycerol Free Taq DNA Polymerase (1000 units) with 10x Key Buffer with 10x Extra Buffer	**733-1817**
VWR Taq DNA Polymerase (500 units) with 10x Key Buffer with 10x Extra Buffer	**733-1301**
VWR Taq DNA Polymerase (500 units) with 10x Extra Buffer (Mg^{2+}-free and Triton-free)	**733-1304**
VWR Taq DNA Polymerase (1000 units) with 10x Key Buffer (Tween-free) with 10x Extra Buffer (Triton-free)	**733-1307**
VWR Taq DNA Polymerase (500 units) with 10x Key Buffer (Mg^{2+}-free)	**733-1311**
VWR Red Taq DNA Polymerase (2500 units) with 10x Key Buffer with 10x Extra Buffer	**733-1323**
VWR Taq DNA Polymerase Master Mix*	
VWR Taq DNA Pol. 1.1x Master Mix, 1.5 mM $MgCl_2$ final conc. (2500 reactions)	**733-1314**
VWR Taq DNA Pol. 2x Master Mix, 1.5 mM $MgCl_2$ final conc. (2500 reactions)	**733-1316**
VWR Red Taq DNA Pol. 1.1x Master Mix, 1.5 mM $MgCl_2$ final conc. (2500 reactions)	**733-1318**
VWR Red Taq DNA Pol. 2x Master Mix, 1.5 mM $MgCl_2$ final conc. (2500 reactions)	**733-1320**
VWR TEMPase Hot Start DNA Polymerase	
VWR TEMPase Hot Start DNA Polymerase (500 units) with 10x Key Buffer with 10x Combination Buffer	**733-1331**
VWR Glycerol Free TEMPase DNA Polymerase (500 units) with 10x Key Buffer	**733-2555**
VWR TEMPase Hot Start Master Mix**	
VWR TEMPase Hot Start 2x Master Mix K, 1.5 mM $MgCl_2$ final conc. (2500 reactions)	**733-2582**
VWR Blue TEMPase Hot Start 2x Master Mix K, 1.5 mM $MgCl_2$ final conc. (2500 reactions)	**733-2585**
VWR Multiplex Master Mix	
VWR Multiplex 2x Master Mix, (2500 reactions)	**733-2569**
VWR High Fidelity DNA Polymerase	
VWR AccuPOL DNA Polymerase (250 units) with 10x Key Buffer	**733-1324**
VWR AccuPOL DNA Polymerase (250 units) with 10x Key Buffer (Mg^{2+} free and Tween free)	**733-1328**
VWR PCR accessories	
Betaine Enhancer Solution 5 M, 5 ml (500 reactions)	**733-1361**
dNTP Mix, 40 mM (10 mM of each dATP, dCTP, dGTP & dTTP) (40 µmol, 2x 500 µl)	**733-1363**
dNTP Set, 100 mM of each dATP, dCTP, dGTP & dTTP (4x 25 µmol, 4x 250 µl)	**733-1364**
VWR PCR grade water (6 x 5 ml)	**733-2573**
VWR Loading Buffer Red (5 x 1 ml) – available in 4 different colors	**733-2574**

*Also available with 2 mM $MgCl_2$
**Also available as 2x Master Mix C based on Combination Buffer

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