

Data sheet

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2X UltraMas^{PLUS}Mix-2025

RESEARCH USE ONLY

Ready-to-use MasterMix for "fast-PCR" and "anti-contamination" PCR

Cat.No.	Pack	Conc.
MMUP-100	100 rnx	2 X
MMUP-500	500 rnx	2 X

DESCRIPTION

2X UltraMas^{PLUS}Mix is a ready-to-use premix of all components for amplification of target DNA, contains **stabilizer/ enhancer**, which improves thermostabilization of enzyme during PCR amplification and storage.

2X UHotMas^{Plus} contains **dUTP, UDG** and **special blend of antibodies-blocked polymerases**, which are not active at ambient temperature (during PCR set-up) and activated automatically during the first PCR cycle at the temperature >70°C, preventing miss-priming and other artifacts formation. In addition,

2X UHotMas^{Plus} prevents formation of false-positive amplicons, due to pre-amplification treatment of reaction mixture by UDG, what allows distracting possible unwanted contaminations.

It is no need for prolonged heating for activation of enzyme for PCR.

2X UltraMas^{PLUS}Mix contains optimized buffer reagents, which greatly improve specificity of PCR with **complex, low-copy number DNA templates, multiplex PCR, "real-time" PCR**, allowing to use very small initial quantities of DNA template.

2X UltraMas^{PLUS}Mix can be used for "fast-PCR" amplification, reducing total reaction time up to 30-35 min. for amplification of DNA targets up to 1Kb.

Total run-time of PCR using **2X UltraMas^{PLUS}Mix** could be dramatically reduced by:

- **shortening of annealing/elongation times up to 5-10 seconds per kilobase of DNA;**
- **combining of Annealing and Elongation steps;**
- **increasing of Denaturation Temperature to 98°C vs. 95°C (for commonly used Polymerases) to overcome problems with DNA secondary structures, and decreasing, at the same time, of the denaturation time up to 1-2 sec. per cycle;**

Recommended PCR assay

50µl PCR assay	Final Conc.
25µl 2X UltraMas^{PLUS}Mix 2025	1X
0.1-1µM each Primer	
Variable* DNA Template	
To 50µl PCR Grade Water	

*- depending on DNA template initial concentration

APPLICATIONS:

- "fast-PCR" amplification
- "anti-contamination" PCR
- "Real-Time" PCR (all types)
- Low-copy PCR (UltraSmarTaq Polymerase)
- Multiplex PCR

Stability:

2X UltraMas^{PLUS}Mix stable for 24 months at -20°C, or for 12 months at +4°C storage without freezing.

CONTENT:

1X: UltraSmarTaq Polymerase

UDG (Uracil DNA Glycosylase)

0.2mM dATP,dCTP,dGTP

0.1mM dTTP,dUTP

2,0 mM MgCL₂

Reaction Buffer components

Stabilizer/enhancer

SHIPPING CONDITIONS:

Should be shipped at ambient temperature

For long distance shipments preferably in

Blue Ice

STORAGE CONDITIONS :

Store **2XUltraMas^{PLUS}Mix** at -20°C (for long-term storage).

General Protocol for amplification with 2X UltraMas^{PLUS}Mix -2025

During PCR amplification, the polymerase in the **2X UltraMas^{PLUS}Mix -2025** amplifies target DNA using sequence-specific primers.

For all pairs -target DNA/primers protocol must be adopted for your specific DNA sample.

2X UltraMas^{PLUS}Mix -2025 provided at a convenient 2X concentration. Only the addition of DNA template of choice and specific primers is necessary.

Add and mix the following components:

Component	50 μ L reactions	25 μ L reactions	Final concentration
PCR grade Water	Up to 50 μ L	Up to 25 μ L	
2X UltraMas^{PLUS}Mix-2025	25 μ L	12.5 μ L	1X
Primers			0.3-0.5 μ M each
Template DNA	optionally	optionally	1-50ng

In some cases, we recommends to optimize Mg concentration in the range 2.5-4.5mM
We recommend using 25 μ l reaction for the PCR with **2X UltraMas^{PLUS}Mix-2025**

Cycling Protocol:

Cycle step	2-Step		3-step amplification		Cycles
	T $^{\circ}$ C	T $^{\circ}$ C	Time		
Pre-amplification treatment	47-50 $^{\circ}$ C	47-50 $^{\circ}$ C	2 min		1
Initial Denaturation	98 $^{\circ}$ C	98 $^{\circ}$ C	1-2 min		1
Denaturation Annealing/ Extension	98 $^{\circ}$ C 66-72 $^{\circ}$ C	98 $^{\circ}$ C 55-66* 72 $^{\circ}$ C	2-10 S 10-15 S 15 Sec/Kb**		30-40
Final extension	72 $^{\circ}$ C	72 $^{\circ}$ C	1-2 min		1
	4 $^{\circ}$ C	4 $^{\circ}$ C	hold		

1. Pre-amplification

Pre-amplification treatment needed for destruction of possible contamination (Uracil – containing amplicons) from the previous amplification by Uracil DNA-Glycosylase.

NOTE: If you used in the other amplification dNTP's not-containing dUTP (only regular dNTP's) UDG treatment is senselessly.

2. Denaturation:

- 1) For most applications time up to 1min of "Initial Denaturation" is quite enough. Impirically it could be reduced up to 30 seconds;
- 2) Initial denaturation at 98 $^{\circ}$ C preferable during PCR amplification with **UltraSmarTaq** Polymerase;
- 3) For most applications, including "direct-blood" 2 sec at 98 $^{\circ}$ C is enough for denaturation of the sample in during PCR run.

NOTE: Do not use lower T denaturation, then 98 $^{\circ}$ C; it can cause problems in PCR (nonspecific amplification, poor yield of PCR product, etc.)

3. Annealing/Extension:

NOTE! 2X UltraMas^{PLUS}Mix -2025 optimized for the annealing/extension parameters for the primers with T_m at or above 60-62 $^{\circ}$ C

For **2 X UltraMas^{PLUS}Mix -2025 "Annealing" and "Extension"**, steps should be combined if:

- T_m of both primers are not differs dramatically (<3 $^{\circ}$ C);
- T_m of the primers are at or above 60-62 $^{\circ}$ C (optimal T_m for the primers lays between 64-68 $^{\circ}$ C)
- If primers T_m is about 60-62 $^{\circ}$ C for both primers, ones can apply simple formula to determine starting Ta/e point - **(T_m of the lower primer +72 $^{\circ}$ C)/2**. As the starting point of determining of Ta/e, see table below. For most applications, it works fine.
- To determine an optimal Ta/e for better amplification results run gradient amplification.

-To avoid nonspecific band formation/smearing during amplification not exceed extension time of 30 seconds and use the highest ramp rate of amplifier (the ramp rate >4-5°C preferable)

**For non-complex DNA templates (plasmid DNA, phage DNA, BAC clone) extension time could be reduced up to 15 sec/Kb.

For complex DNA templates (human DNA) in amplification of the fragments longer than 2Kb strongly recommended to apply **Extension time** as 30 sec/Kb

For the DNA templates with GC content less 65% and up to 500bp length, it is quite enough to set Extension time as 15 sec per cycle.

For 2-step amplification it is better to combine Annealing and Extension steps and to use whole time of the combined step **10-15 sec for the targets <1500 bp.**

3. Final Extension:

For most applications 30 seconds of "Final Extension" step is enough. If you'll use amplification product for further cloning – it's better to use prolonged final extension up to 3 min.

Optimization of Cycling Conditions

Primers Tm calculated	Ta/e, °C	GC- content,%	Elongation,sec	Cycles No	Target size, bp
60-62	64-66	High >55%	10-15	30-35	<500
		Low<55%	5-10		
63-65	67-69	High >55%	10-15	30-35	<500
		Low<55%	5-10		
67-69	70-72	High >55%	10-15	30-40	<500
		Low<55%	5-10		
69-72	70-72	High >55%	10-15	30-40	<500
		Low<55%	5-10		

All data in the table are only recommendation and starting point.

To get optimal results, cycling conditions for every primers pair must be optimized, depending on initially calculated Tm of primers, GC-content, target length, specificity and other crucial for amplification parameters.