

Data sheet

This data sheet provided for information only

2X UltraMas^{CF}Mix-2025**RESEARCH USE ONLY****Ready-to-use MasterMix for "fast-PCR" amplification**

Cat.No.	Pack	Conc.	DESCRIPTION
MMUS-100	100 rnx	2 X	2X UltraMas^{CF}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.
MMUS-500	500 rnx	2 X	

Stability:

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

CONTENT:**1X: UltraSmarTaq** Polymerase**0.2mM** each of dNTP's**2,0 mM** MgCL₂

Reaction Buffer components

Stabilizer/enhancer

2X UltraMas^{CF}Mix contains **UltraSmarTaq Polymerase - 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.

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

2X UltraMas^{CF}Mix can be used for "fast-PCR" amplification, reducing total reaction time up to 25-35 min. for amplification of DNA targets up to 1Kb.

Total run-time of PCR using **2X UltraMas^{CF}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;

2X UltraMas^{CF}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.

Recommended PCR assay

50µl PCR assay		Final Conc.
25µl	2X UltraMas^{CF}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

- Primer extension

- "Real-Time" PCR (all types)

- Low-copy PCR (UltraSmarTaq Polymerase)

- Multiplex PCR

SHIPPING CONDITIONS:

Should be shipped at ambient temperature.

For long distance shipments preferably in

Blue Ice

STORAGE CONDITIONS :

Store **2X UltraMas^{CF}Mix** at -20°C (for long-term storage).

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

During PCR amplification, the polymerase in the **2X UltraMas^{CF}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^{CF}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^{CF}Mix-2025	25 μ L	12.5 μ L	1X
Primers			0.2-0.5 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^{CF}Mix-2025**

Cycling Protocol:

Cycle step	2-step amplification		Cycles
	T ^o C	Time	
Initial Denaturation	98^oC	1-2 min	1
Denaturation	98^oC	1-5 S	30-40
Annealing/ Extension	*62-72 ^oC	5-15 S	
Final extension	72^oC 4^oC	0,5-3 min hold	1

1. 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 for 5 min at 98^oC is necessary only for blood cells lysis;

-3) For most applications, including "direct-blood" 2 sec at 98^oC is enough for denaturation of the sample in during PCR run.

NOTE: Don't use lower T denaturation, then 98^oC; it can cause problems in PCR (nonspecific amplification, poor yield of PCR product, etc.)

2. Annealing/Extension:

NOTE! 2X UltraMas^{CF}Mix -2025 optimized for the annealing/extension parameters for the primers with T_m at or above 60-62^oC

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

- T_m of both primers are not differs dramatically (<3^oC);

- T_m of the primers are at or above 60-62^oC (optimal T_m for the primers lays between 64-68^oC)

- If primers T_m is about 60-62^oC for both primers, ones can apply simple formula to determine starting Ta/e point - **(T_m of the lower primer +72^oC)/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 amplificator (the ramp rate >4-5^oC 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's quite enough to set Extension time as 15 sec per cycle.

3. Final Extension:

For most applications 30 seconds of "Final Extension" step is enough. If you'll use amplification product for further cloning – it is 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% Low <55%	10-15 5-10	30-35	<500
63-65	67-69	High >55% Low <55%	10-15 5-10	30-35	<500
67-69	70-72	High >55% Low <55%	10-15 5-10	30-40	<500
69-72	70-72	High >55% Low <55%	10-15 5-10	30-40	<500

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.