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Data sheet
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2X MultiMas^{CF}Mix-4035

RESEARCH USE ONLY

Ready-to-use MasterMix for "fast-PCR" multiplex amplification

Cat.No.	Pack	Conc.
MMUS-100	**100 rnx**	**2 X**
MMUS-500	**500 rnx**	**2 X**

Stability:
2X MultiMas^{CF}Mix stable for 24 months at -20°C, **or** for 6 months at +4°C storage without freezing.

CONTENT:
1X: UltraSmarTaq Polymerase
0.2mM each of dNTP's
2,0 mM $MgCL_2$
Reaction Buffer components
Stabilizer/enhancer

DESCRIPTION
2X MultiMas^{CF}Mix is a ready-to-use premix of all components for amplification (excluding primers and DNA template) of target DNA, contains **stabilizer/ enhancer,** which improves thermostabilization of enzyme during PCR amplification and storage**.**
2X MultiMas^{CF}Mix designed for amplification of multiplex DNA targets in single tube, providing highly specific PCR with highest yields of products.
2X MultiMas^{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 MultiMas^{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 MultiMas^{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 MultiMas^{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 MultiMas^{CF}Mix 4035**	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 **2XMultiMas^{CF}Mix** at -20°C (for long-term storage).

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General Protocol for amplification with 2X MultiMas^{CF}Mix -4035

During PCR amplification, the polymerase in the **2X MultiMas^{CF}Mix -4035** amplifies target DNA using sequence-specific primers.
For all pairs -target DNA/primers protocol must be adopted for your specific DNA sample.
2X MultiMas^{CF}Mix -4035 provided at a convenient 2× concentration. Only the addition of DNA template of choice and specific primers is necessary.

Add and mix the following components:

Component	50μL reactions	25μL reactions	Final concentration
PCR grade Water	Up to 50 μL	Up to 25 μL	
2XMultiMas^{CF}Mix-4035	25 μL	12.5 μL	1X
Primers (pair #1)			0.2-1 μM each*
Primers (pair #2)			0.2-1 μM each
Primers (pair #3)			0.2-1 μM each
Primers (pair #4)			0.2-1 μM each
Primers (pair #...)			0.2-1 μM each
Template DNA	optionally	optionally	10-50ng

For most applications, it will fine to start with amplification of the single DNA Targets for all primer pairs to determine the best initial concentrations of primers. Usually at the first step, concentrations of primers are equimolar.
After first step – combine all primers and run PCR. If result is not fits you (low yields of some products of interest/some targets not amplified), increase primers concentration for the targets, which are not/poor amplified and repeat amplification with another concentrations of primers.
Additional optimization of the ratio of primers in the mix might be very useful to get optimal result.
In some cases, we recommends to optimize Mg concentration in the range 2.5-4.5mM
We recommend using 25μl reaction for the PCR with **2X MultiMas^{CF}Mix-4035**

Cycling Protocol:

Cycle step	2-step amplification		Cycles
	T°C	Time	
Initial Denaturation	**98°C**	**1-2 min**	**1**
Denaturation **Annealing/ Extension**	**98°C** ***62-72 °C**	**1-5 S** **5-15 S**	**30-40**
Final extension	**72°C** **4°C**	**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) for most applications, including "direct-blood" 2 sec at 98°C is enough for denaturation of the sample in during PCR run.
NOTE: Don't use lower T denaturation, then 98°C; it can cause problems in PCR (nonspecific amplification, poor yield of PCR product, etc.)

2.Annealing/Extension:

NOTE! 2X MultiMas^{CF}Mix -4035 optimized for the annealing/extension parameters for the primers with Tm at or above 60-62°C

For **2 X MultiMasCFMix -4035 "Annealing" and "Extension",** steps should be combined if:
- Tm of both primers are not differs dramatically (<3°C);
- Tm of the primers are at or above 60-62°C (optimal Tm for the primers lays between 64-68°C)
- If primers Tm is about 60-62°C for both primers, ones can apply simple formula to determine starting Ta/e point **- (Tm of the lower primer +72°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.

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-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 >4-5ºC 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, ºc	GC- content,%	Elongation,sec	Cycles No	Target size, bp
60-62	66-68	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.

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