



**FAQ's about the Failsafe™PCR System and MasterAmp™
Extra Long PCR**

**Click here for FAQ's about Failsafe™
PCR system**

**Click for here FAQ's about MasterAmp™
extra long PCR**

FailSafe™ PCR System

How does FailSafe work?

Can you amplify Fragile X genes (the GCC-repeat) with FailSafe?

Can I order the FailSafe enzyme without the PCR PreMixes?

How long does it take to optimize with FailSafe?

How many “reactions” do I get with the FailSafe PCR PreMix Selection Kit?

If I get my reaction to work with a specific PreMix on the first try, how many PreMix-specific reactions can I get out of the FailSafe PreMix Selection Kit?

What are the compositions of each of the PreMixes?

What kind of enzyme is the FailSafe DNA Polymerase?

Can FailSafe be used for Real-time and quantitative PCR?

**Are there any literature references available for FailSafe? My boss won't let me buy it unless there are some scientific journal citations published.
What is the longest template I can amplify?**

Does a FailSafe PCR product have A-tailed 3'-ends?

Can I use FailSafe, along with a reverse transcriptase, for RT-PCR?

Can you tell me which FailSafe PreMix I should use for my template? It is GC-rich. I don't want to do all of the 12 PreMixes because it takes too much time.

Can I scale up my FailSafe PCR to a larger volume? It's working great for my template and primers at 50 µl reaction volume.

I want to amplify a 20-kb template. Which one of your PCR Kits should I use?

Can I use FailSafe with a carryover prevention system? (using dUTP and UNG to prevent PCR template contamination in the lab).

Can I use Topo or TA-cloning with the PCR product from the FailSafe PCR System?

**Cambio Ltd, 1 The Irwin Centre, Scotland Road, Dry Drayton, Cambridge, CB23 8AR, UK
Tel :+44 (0)1954 210 200 , +44 (0)1954 210 300, email: support@cambio.co.uk
www.cambio.co.uk**

I want to use FailSafe with random hexamer primers to get a wide variety of amplification products. Can you give me a suggested cycling procedure?

Can I use the FailSafe PCR System in a Taqman assay?

How easy is it to optimize a multiplex PCR using FailSafe?

I have a set of primers for a gene has an intron in it, but I don't know how long the full-length (intron included) product is supposed to be. Can I still use FailSafe? What would the best conditions be for trying to get the full-length gene if I don't know the expected size?

My PCR still won't work. Why? I thought you said it was "Failure-proof"

I used FailSafe and got a weak band instead of the one strong band you promised. Why is that? What can I do about it?

I'm getting multiple bands with my FailSafe PCR. Why? How can I fix it?

I tried all of your suggestions and I still can't get my FailSafe PCR System to work. Now, what do I do?

I'm trying to amplify a closed circular plasmid but I get many different bands using FailSafe. Why is this and how do I fix it?

I'm trying to do inverse PCR of a closed circular plasmid, and all I get is a smear even with FailSafe. Can you help me fix this?

How does FailSafe work?

The FailSafe Enzyme Mix produces a high fidelity PCR product. The twelve individual FailSafe PCR PreMixes contain all you need for PCR, and they contain varying amounts of Magnesium and FailSafe PCR Enhancer. Perform 12 reactions, analyze on an agarose gel, and choose the PreMix that gives you the best PCR product. Use that PreMix for all PCRs with that primer pair.

Can you amplify Fragile X genes (the GCC-repeat) with FailSafe?

Yes, call or e-mail Fred or Hank for details.

Can I order the FailSafe enzyme without the PCR PreMixes?

Yes, this is possible, contact Fred, Hank or Customer Service for details.

How long does it take to optimize with FailSafe?

As fast as you can do a PCR reaction and perform agarose gel electrophoresis.

How many “reactions” do I get with the FailSafe PCR PreMix Selection Kit?

8, 50-ul reactions, or enough to optimize 4 primer-template combinations. More reactions can be obtained using a lower reaction volume, but more enzyme will be needed (see above on ordering FailSafe PCR Polymerase without the PreMixes)

If I get my reaction to work with a specific PreMix on the first try, how many PreMix-specific reactions can I get out of the FailSafe PreMix Selection Kit?

Eight, 50-ul reactions.

What are the compositions of each of the PreMixes?

They contain defined amounts of Salt, Buffer, and all four dNTPS – they contain proprietary and varying amounts of Magnesium and PCR Enhancer.

What kind of enzyme is the FailSafe DNA Polymerase?

This is proprietary and we cannot tell you- sorry

Can FailSafe be used for Real-time and quantitative PCR?

Yes.

Are there any literature references available for FailSafe? My boss won't let me buy it unless there are some scientific journal citations published.

There are several, but there are rarely, if ever, any specifics for cycling conditions or which PreMix was used (a typical citation: “The H. ducreyi lgtA gene was amplified by PCR using the FailSafe PCR PreMix selection kit (Epicentre Technologies). The pair of oligonucleotide primers were targeted to a

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Tel :+44 (0)1954 210 200 , +44 (0)1954 210 300, email: support@cambio.co.uk
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1.9-kb DNA fragment containing the *lgtA* gene and the majority of the *pfkA* gene (Fig. 2). The two oligonucleotide primers were GlcNAc-F (5'-CTCGGAAATTATTAACCGTGGTGGTAC-3') and GlcNAc-R (5'-AGCGGTTATTAATGTAAATAACAGACGG-3').

What is the longest template I can amplify?

Anything up to 20 kbp is fine, for longer PCRs use MasterAmp Extra-Long PCR Kit.

Does a FailSafe PCR product have A-tailed 3'-ends?

Yes.

Can I use FailSafe, along with a reverse transcriptase, for RT-PCR?

Yes.

Can you tell me which FailSafe PreMix I should use for my template? It is GC-rich. I don't want to do all of the 12 PreMixes because it takes too much time.

You must perform the full PreMix selection procedure. There is no magic formula that will tell you the best PreMix to use on a given primer/template combination. If your template is limited by the amount you have contact Fred or Hank for help.

Can I scale up my FailSafe PCR to a larger volume? It's working great for my template and primers at 50 µl reaction volume.

Yes – and we recommend multiple PCRs of 100 µl for scale-up.

I want to amplify a 20-kb template. Which one of your PCR Kits should I use?

Use MasterAmp Extralong PCR Kit.

Can I use FailSafe with a carryover prevention system? (using dUTP and UNG to prevent PCR template contamination in the lab).

No. The proofreading portion of the FailSafe Polymerase will prevent incorporation of dUTP.

Can I use Topo or TA-cloning with the PCR product from the FailSafe PCR System?

Yes. The FailSafe PCR Enzyme Mix adds an uncoded "A" to both ends of many of the PCR products, and thus can be used in TA-cloning applications using T-vectors and also with Topo-TA cloning products.

I want to use FailSafe with random hexamer primers to get a wide variety of amplification products. Can you give me a suggested cycling procedure?

Typically, you would set the anneal temperature for the primers to be quite low. This will not hurt the FailSafe Polymerase. As nucleotides are added the “melting” temperature of the extended primer goes up and better “locks” the primer to the template until the next denaturation step.

Can I use the FailSafe PCR System in a Taqman assay?

We have reports from our customers that Failsafe is compatible with TaqMan. Please contact technical Support.

How easy is it to optimize a multiplex PCR using FailSafe?

Very easy. Optimizing multiplex PCR is done the exact same way as with a single template/primer system.

I have a set of primers for a gene has an intron in it, but I don't know how long the full-length (intron included) product is supposed to be. Can I still use FailSafe? What would the best conditions be for trying to get the full-length gene if I don't know the expected size?

This can be a bit tricky, and will require a bit of “playing around” with the extension time. If you have some knowledge of the expected size of the “un-processed” template (gene plus intron), that will help considerably. If you don't, then you should start out by optimizing the “processed” gene using FailSafe. Once you have the basic cycling conditions you can continue by doing FailSafe and lengthening the extension time in varying increments until you start to see a longer band forming.

Typical calls from people who are having trouble:

Technical support for more information .

My PCR still won't work. Why? I thought you said it was “Failure-proof”?

The usual problems (99%) are poor primer design (inaccurate T_m measurements, primer dimer, primer pair T_ms too far apart or cycling conditions poorly selected. Review the caller's information in as much detail as possible and have them contact Hank or Fred directly.

I used FailSafe and got a weak band instead of the one strong band you promised. Why is that? What can I do about it?

Increase the number of cycles, increase (slightly) the amount of template you are using.

I'm getting multiple bands with my FailSafe PCR. Why? How can I fix it?

Pseudogenes, poorly selected cycling conditions, poorly designed primers leading to low specificity primer design.

I tried all of your suggestions and I still can't get my FailSafe PCR System to work. Now, what do I do?

Contact Technical support and they will put you in touch with the experts.

I'm trying to amplify a closed circular plasmid but I get many different bands using FailSafe. Why is this and how do I fix it?

The supercoiling has the effect of blocking the processive nature of the polymerase and topological "knots" can cause real problems for the polymerase staying on the template. Use either topoisomerase or linearize the template if at all possible. If using topo, monitor the plasmid relaxation on a gel.

I'm trying to do inverse PCR of a closed circular plasmid, and all I get is a smear even with FailSafe. Can you help me fix this?

See above

MasterAmp™ Extra-Long PCR Kit

Similar in format to the FailSafe system, the MasterAmp Extra-Long PCR Kit uses three fewer PCR PreMixes and is able to amplify very long templates (~41 kb). The Extra-Long Polymerase has excellent proofreading capabilities and is able to get through templates with very tough GC-rich regions.

How does your kit compare to other “extra long” kits, such as Takara’s LA-PCR, Roche’s “EXPAND”, etc.?

How does your extra-long PCR Kit work?

I want to amplify a template that’s around 20-22 kb. I see you have the FailSafe PCR System and the MasterAmp Extra-Long PCR Kit. Which should I use?

How does the MasterAmp PCR Optimization kit work?

Are the PCR PreMixes the same as in the FailSafe PCR System?

I’m currently using FailSafe and get a pretty good PCR product. Can I use the MasterAmp PCR Optimization Kit to make it even better?

What are the main differences between the MasterAmp PCR Optimization Kits with and without Ammonium Sulfate?

How does the Ammonium sulfate make PCR better?

What is in the MasterAmp 10X PCR Enhancer?

Can I use the Enhancer with PCR kits that are not sold by Epicentre?

Will the MasterAmp PCR Enhancer help me with a tough RT-PCR?

I was using your Enhancer successfully but it began to fail about a year after I bought it. Does it have an expiration date? How long should it be good for?

How does your kit compare to other “extra long” kits, such as Takara’s LA-PCR, Roche’s “EXPAND”, etc.?

Mass of PCR product will be better with the Epicentre Kit, and optimization is greatly simplified with the Epicentre Kit.

How does your extra-long PCR Kit work?

The optimization protocol is identical to the FailSafe product, with the exception that the MasterAmp Extra-Long PCR Kit has only nine “PreMixes”.

I want to amplify a template that’s around 20-22 kb. I see you have the FailSafe PCR System and the MasterAmp Extra-Long PCR Kit. Which should I use?

Use the MasterAmp Extra-Long PCR Kit.

How does the MasterAmp PCR Optimization kit work?

Essentially the same as the FailSafe PCR Selection Kit – see above. The exception is that the customer will not be using the FailSafe PCR Enzyme Mix.

Are the PCR PreMixes the same as in the FailSafe PCR System?

No.

I’m currently using FailSafe and get a pretty good PCR product. Can I use the MasterAmp PCR Optimization Kit to make it even better?

No.

What are the main differences between the MasterAmp PCR Optimization Kits with and without Ammonium Sulfate?

The kit with Ammonium Sulfate is used with Tfl, Tth and all proofreading thermostable DNA Polymerases, the kit without Ammonium Sulfate is used with Taq DNA Polymerase(s).

How does the Ammonium sulfate make PCR better?

The Ammonium Sulfate makes is necessary for optimal ‘proofreading’.

What is in the MasterAmp 10X PCR Enhancer?

It is a mixture of Betaine (to which Epicentre has the exclusive rights for its patented use in PCR) and other proprietary components.

Can I use the Enhancer with PCR kits that are not sold by Epicentre?

Yes, with excellent effect.

Will the MasterAmp PCR Enhancer help me with a tough RT-PCR?

Very likely, yes, mostly with the PCR portion of the reaction. The effect of the Enhancer on reverse transcription with either MMLV or a “RetroAmp”-type of thermostable RT is not clear.

I was using your Enhancer successfully but it began to fail about a year after I bought it. Does it have an expiration date? How long should it be good for?

If stored at -20°C, it will remain stable for years.