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FailSafe™ PCR PreMix Selection Kit
FailSafe™ PCR System with PreMix Choice
FailSafe™ Enzyme Mix Only
FailSafe™ PCR 2X PreMixes

Cat. Nos. FS99060, FS99100, FS99250, FS9901K, FSE51100, FSE5101K, and FSP995A→L

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1. Introduction

The FailSafe™ PCR PreMix Selection Kit*† contains a unique blend of thermostable DNA polymerases and a set of twelve reaction PreMixes. This comprehensive set of reagents was specifically designed to meet every PCR need. Any template: routine; difficult (e.g., high GC content or secondary structure); or long (approximately 20 kb in length), can easily be amplified with FailSafe PCR. And because the FailSafe PCR Enzyme Mix provides fidelity at least three times higher than *Taq* DNA polymerase alone, PCR products are suitable for downstream applications such as cloning, sequencing, expression, and mutation analysis.

The FailSafe PCR Enzyme Mix is an enzyme blend containing a 3′→5′ proofreading enzyme for high fidelity. The 12 FailSafe PCR 2X PreMixes contain a buffered salt solution with all 4 dNTPs, and various amounts of $MgCl_2$, and FailSafe PCR Enhancer (with betaine).+ The user simply adds template, primers, and the FailSafe PCR Enzyme Mix to each of the PreMixes and amplifies. The results will clearly show which PreMix is best for that template/primer pair combination. The presence of betaine (trimethyl glycine) in the FailSafe PCR Enhancer substantially improves the yield and specificity of amplification of many target sequences, especially those containing a high G+C content or secondary structure.[1-5] In addition, betaine also may enhance PCR by protecting DNA polymerases from thermal denaturation.[6] The effects of betaine seem to be independent of the polymerase used, though the concentration of betaine required for amplification varies with the target sequence.[1-4]

Once the optimal PreMix has been determined, consistent amplification is achieved with the FailSafe™ PCR System with PreMix Choice*† customized to your template/primer pair combination. Choose from 3 different sizes of FailSafe PCR Enzyme Mix paired with the FailSafe PCR PreMixes of your choice. Individual FailSafe™ PCR 2X PreMixes and FailSafe™ Enzyme Mix Only*† are also available separately.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Note: *Some of the PreMixes may not freeze completely.*

Storage Buffer: The FailSafe PCR Enzyme Mix is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.5% Tween®20, 0.5% NP-40, and 1 mM dithiothreitol.

FailSafe PCR 2X PreMixes: The FailSafe PCR 2X PreMixes contain 100 mM Tris-HCl (pH 8.3), 100 mM KCl, and 400 µM of each dNTP. The concentrations of $MgCl_2$ (3-7 mM) and FailSafe PCR Enhancer (0-8X) vary with the individual mixes.

Unit Definition: One unit converts 10 nmoles of deoxyribonucleoside triphosphates into acid-insoluble material in 30 minutes at 70°C using standard assay conditions.

Quality Control: FailSafe PCR PreMix Selection Kit, FailSafe PCR System and individual FailSafe PCR 2X PreMixes are function-tested in PCR reactions using the following "difficult" templates: human *Apo*E gene segment, human FRM1 gene segment (≥ 80% G+C) and a 20-kb lambda phage genome segment.

Contaminating Activity Assays: FailSafe PCR Enzyme Mix and individual FailSafe PCR 2X PreMixes are free of detectable nonspecific DNase and RNase activities as judged by agarose gel electrophoresis following over-digestion assays.

Activity Assay: The activity assay is performed in a reaction containing 25 mM TAPS (pH 9.3), 50 mM KCl, 2.0 mM $MgCl_2$, 8.5 µg of activated calf thymus DNA, 0.2 mM of each dNTP, and 0.02-0.1 unit of enzyme.

3. Kit Contents

Cat. #		Concentration	Quantity
FailSafe™ PCR Components			
FS99060	**FailSafe PCR PreMix Selection Kit**		
	FailSafe PCR Enzyme Mix	@ 2.5 U/µl	60 U
	FailSafe PCR 2X PreMixes	A through L	each 100 µl
Note: *Each PreMix volume has been modified to match the Enzyme Mix volume.*			
FS99100	**FailSafe PCR System with PreMix Choice**		
	FailSafe PCR Enzyme Mix	@ 2.5 U/µl	100 U
	Choice of 1 FailSafe PCR 2X PreMix	2.5 ml	
FS99250	FailSafe PCR Enzyme Mix	@ 2.5 U/µl	250 U
	Choice of 2 FailSafe PCR 2X PreMixes	each 2.5 ml	
FS9901K	FailSafe PCR Enzyme Mix	@ 2.5 U/µl	1,000 U
	Choice of 8 FailSafe PCR 2X PreMixes	each 2.5 ml	
FailSafe Enzyme Mix Only**			
FSE51100	FailSafe PCR Enzyme Mix	@ 2.5 U/µl	100 U (40 µl)
FSE5101K	FailSafe PCR Enzyme Mix	@ 2.5 U/µl	1,000 U (400 µl)

Individual FailSafe PCR 2X PreMixes

FSP995A – FailSafe PCR 2X PreMix A	2.5 ml
FSP995B – FailSafe PCR 2X PreMix B	2.5 ml
FSP995C – FailSafe PCR 2X PreMix C	2.5 ml
FSP995D – FailSafe PCR 2X PreMix D	2.5 ml
FSP995E – FailSafe PCR 2X PreMix E	2.5 ml
FSP995F – FailSafe PCR 2X PreMix F	2.5 ml

***** **Note:** *Epicentre can only guarantee the "failsafe" nature of this system if the FailSafe Enzyme Mix is used with a FailSafe PCR 2X PreMix that is selected using the FailSafe PCR PreMix Selection Kit.*

*,+,† *See page 9 for patent and licensing information.*

4. Related Products

The following products are also available:

– TAQXpedite™ PCR System (FAST end-point)

– MasterPure™ Nucleic Acid Purification Kits

– MasterAmp™ Buccal Swab DNA Extraction Kits

– BuccalAmp™ DNA Extraction Kits

5. General Considerations

1. **Template:** DNA prepared using standard isolation techniques is a suitable substrate for amplification. Nevertheless, numerous compounds inhibit amplification including ionic detergents, some gel loading dyes, phenol, and hemin. When purifying templates from agarose gels, minimize exposure to UV irradiation to prevent formation of pyrimidine dimers. Assembly of reactions in a clean area or using positive displacement pipettors with aerosol-barrier tips will minimize the risk of contamination from extraneous DNA templates. The optimal amount of template for a single-copy gene is between 10^4-10^6 copies (i.e., approximately 0.1-10 ng of *E. coli* genomic DNA), though this may vary depending on the source and quality of the template.[7,8]
2. **Primer Design:** Primers typically are 15-30 bases in length and contain approximately 50% G+C residues; the annealing temperatures of primer pairs should be nearly identical. Care must be taken to design primers that do not form hairpin loop structures or are self-complementary. The 5′ end of a primer may contain bases that are not complementary with the template; however, the 3′ end of the primer must be complementary with the template.
3. **Reaction Components:** The ratio of primer to template is important for controlling the specificity and efficiency of amplification; an excess of primer ensures that the denatured template molecules bind to the primers instead of binding to the complementary DNA strand.[9] Nevertheless, too much primer may lead to the formation of nonspecific products or primer dimers.
4. **Cycling Parameters:** Many parameters influence both the specificity and efficiency of amplification including the temperature and duration of denaturation, annealing and elongation, ramp speed, and total cycle number. Amplification beyond ~10^{12} molecules may also result in the appearance of nonspecific products; if the starting number of template molecules is 10^5, 28-30 cycles will yield 10^{12} molecules.[7] Additional variations such as use of a hot start[10] or touchdown/stepdown PCR[11,12] can dramatically improve specificity and yield (see page 6 Troubleshooting Amplification Reactions).
5. **Cloning PCR Products:** PCR products generated with the FailSafe PCR Enzyme Mix have blunt-ends or a non-template encoded adenine residue at the 3′ end. Hence, an efficient number of clones can be obtained with either blunt-end cloning or TA Cloning® strategies.

1. Add a FailSafe Master Mix containing enzyme, template, and primers to each of the 12 FailSafe PCR PreMixes (A-L).
2. Perform PCR.
3. Analyze PCR results and choose the best FailSafe PCR PreMix for subsequent amplifications (see Fig. 2).
4. Repeat Steps 1-3 for each new or troublesome template/primer pair or order additional FailSafe PCR Enzyme Mix and specify the desired FailSafe 2X PreMix(es).

A 75% GC-rich region of the human *Apo*E gene was amplified. M, molecular marker; Lane 1, standard buffer conditions using MasterAmp *Taq* DNA polymerase; Lanes A-L, amplification with each of the 12 FailSafe 2X PCR PreMixes.

Twelve repeat amplification reactions of the *Apo*E gene from Fig. 2 were performed using FailSafe 2X PCR PreMix K. M, molecular marker.

6. Suggested PCR Protocol

Use the following protocol as a guideline for establishing the parameters necessary for amplification of experimental templates. Minimize contamination risk from extraneous DNA templates by working in a clean area and using positive displacement pipettors with aerosol-barrier tips.

Assemble Amplification Reactions:

The volumes listed below are for one 50-µl amplification reaction. Reaction volumes can be scaled up or down as needed. Assemble an amount of FailSafe Master Mix corresponding to the total number of reactions. Extra Master Mix may be required to offset losses caused by pipeting.

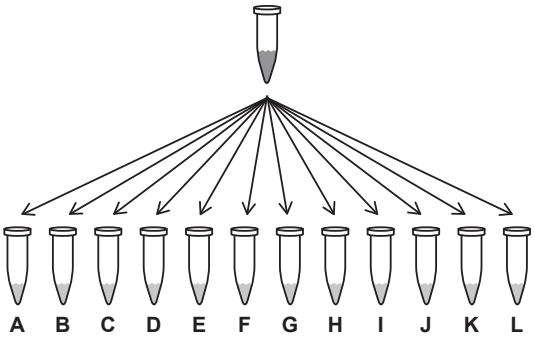

Figure 1. An overview on how to use the FailSafe™ PCR Selection Kit.

1. Prepare the FailSafe Master Mix. Thaw and thoroughly mix all of the reagents listed below before dispensing; place on ice. Combine on ice, all of the following:

x	µl	sterile water
0.2-1.0	µl	50 µM primer 1 (0.2-1 µM final concentration)
0.2-1.0	µl	50 µM primer 2 (0.2-1 µM final concentration)
x	µl	DNA Template (1-500 ng, 10^4-10^6 molecules)

If amplification ≤10 kb:

0.5	µl	FailSafe PCR Enzyme Mix (1.25 Units)

If amplification >10 kb:

1.0	µl	FailSafe PCR Enzyme Mix (2.5 Units)
25	µl	Total reaction volume

2. On ice, aliquot 25 µl of each FailSafe PCR 2X PreMix into an individual PCR tube.
3. Add 25 µl of the FailSafe Master Mix from Step 1 to the PCR tubes and mix.

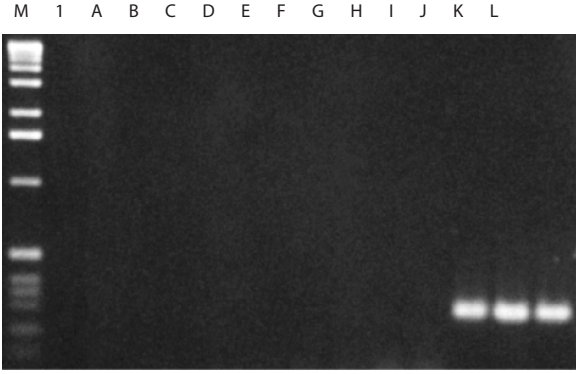

Figure 2. **Amplification of a GC-rich template using the FailSafe™ PCR PreMix Selection Kit.**

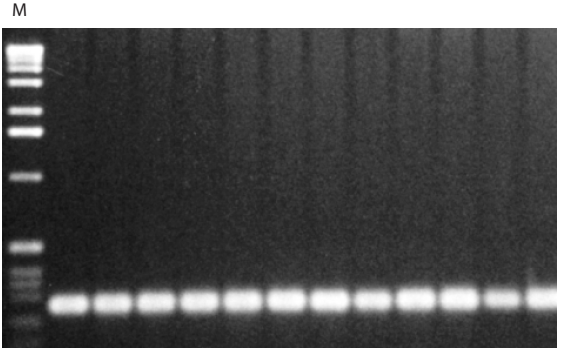

Figure 3. **Consistent amplification with FailSafe™ PCR PreMix K.**

4. Program the thermal cycler following the recommendations provided by the manufacturer.

   A suggested program is outlined below. We recommend a 2-step cycling program for primers with a $T_m \geq 65°C$.

   a) Initially denature the template at 92-98°C for 1-2 minutes.

   b) Perform a 2- or 3-step cycling program, for 20-40 cycles as required:

   Note: *for a 2-step cycling profile, omit the "Anneal" step below.*

   Denature at 92°C-95°C for 0.5-1 minutes.

   Anneal the primers at a temperature 2-5°C below the T_m of the primers for 0.5-1 minute.

   Extend the annealed primers at 68-72°C for 1 minute for every kb of expected product.

5. Place the tubes in the thermal cycler and begin cycling.
6. After amplification, the samples may be kept at 4°C overnight or frozen at –20°C.

7. Troubleshooting Amplification Reactions

Little or no amplification detected

1) **Lower annealing temperature.** Lower the annealing temperature in 2°C increments.
2) **Perform hot start.**[10] The final assembly of amplification reactions at temperatures above the reaction annealing temperature improves both the yield and specificity. Assemble the reactions without the primers; subsequently place the reactions in a thermal cycler heated to >80°C, then add the appropriate amount of each primer and begin the cycling protocol. Alternatively, a modified hot start may be performed in which reactions assembled on ice are added directly to a thermal cycler pre-heated to 92°C-98°C.
3) **Perform Touchdown (TD)/Stepdown (SD) PCR.**[7,11,12] TD or SD PCR consists of a series of cycles that are performed at decreasing annealing temperatures. This protocol favors amplification of the target at temperatures greater than or equal to the optimum annealing temperature, while enhancing target yield in later cycles at annealing temperatures below the T_m of the reaction. Perform the initial series of cycles at an annealing temperature a few degrees above the calculated T_m of the primers; annealing temperature is lowered by 1-4°C every other cycle to ~10°C below the calculated T_m.

   A hot start must be performed if using a TD or SD cycling protocol.
4) **Increase initial template denaturation time or temperature.** Increase the temperature of initial denaturation up to 98°C. Increase the length of initial template denaturation up to 5 minutes. Alternatively, denature the template by heating at 72°C for 10 minutes in the presence of 50 mM NaOH before amplification.
5) **Redesign primers.** Design primers that have higher annealing temperatures and do not form hairpin loops or primer dimers.

6) **Increase number of cycles.** Perform additional cycles in increments of 5.
7) **Vary reaction components.** Vary the amount of DNA polymerase and primers.
8) **Check template quantity and quality.** Check the concentration of template DNA by agarose gel electrophoresis or fluorimetry. Use 10^4-10^6 molecules of template for each reaction (e.g., up to nanogram amounts for cloned templates or microgram amounts for genomic DNA).[7,12] Organic extraction followed by ethanol precipitation may remove some inhibitors of amplification.
9) **Increase extension time.** Increase the extension time, generally 1 minute for every kilobase of product.

Multiple products or a smear detected

1) **Decrease concentration of reaction components.** Check the concentration of template DNA by agarose gel electrophoresis or fluorimetry. Use 10^4-10^6 molecules of template for each reaction (e.g., up to nanogram amounts for cloned templates or microgram amounts for genomic DNA).[7,12] Decrease the amount of enzyme and/or primer added to the reaction.
2) **Increase annealing temperature.** Increase the annealing temperature in 2°C increments.
3) **Perform hot start.**[10] The final assembly of amplification reactions at temperatures above the reaction annealing temperature improves both the yield and specificity. Assemble the reactions without the primers; subsequently place the reactions in a thermal cycler heated to >80°C, then add the appropriate amount of each primer and begin the cycling protocol. Alternatively, a modified hot start may be performed in which reactions assembled on ice are added directly to a thermal cycler pre-heated to 92°C-98°C.
4) **Perform Touchdown (TD)/Stepdown (SD) PCR.**[7,11,12] TD or SD PCR consists of a series of cycles that are performed at decreasing annealing temperatures. This protocol favors amplification of the target at temperatures greater than or equal to the optimum annealing temperature, while enhancing target yield in later cycles at annealing temperatures below the T_m of the reaction. Perform the initial series of cycles at an annealing temperature a few degrees above the calculated T_m of the primers; annealing temperature is lowered by 1°C-4°C every other cycle to ~10°C below the calculated T_m.
   A hot start must be performed if using a TD or SD cycling protocol.
5) **Redesign primers.** Design primers that have higher annealing temperatures and do not form hairpin loops or primer dimers.
6) **Check primers for degradation.** Check by electrophoresis in a denaturing acrylamide gel.
7) **Decrease number of cycles.** Decrease number of cycles in increments of five.

8. References

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