

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**

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 MgCl₂, 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 **Fail-Safe™ 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.

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 MgCl₂ (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 ApoE 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 MgCl₂, 8.5 μg of activated calf thymus DNA, 0.2 mM of each dNTP, and 0.02-0.1 unit of enzyme.

^{*,†} See page 8 for patent and licensing information.

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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 $\sim 10^{12}$ molecules may also result in the appearance of non-specific 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 start¹⁰ or touchdown/stepdown PCR^{11,12} can dramatically improve specificity and yield (see page 4 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.

References:

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

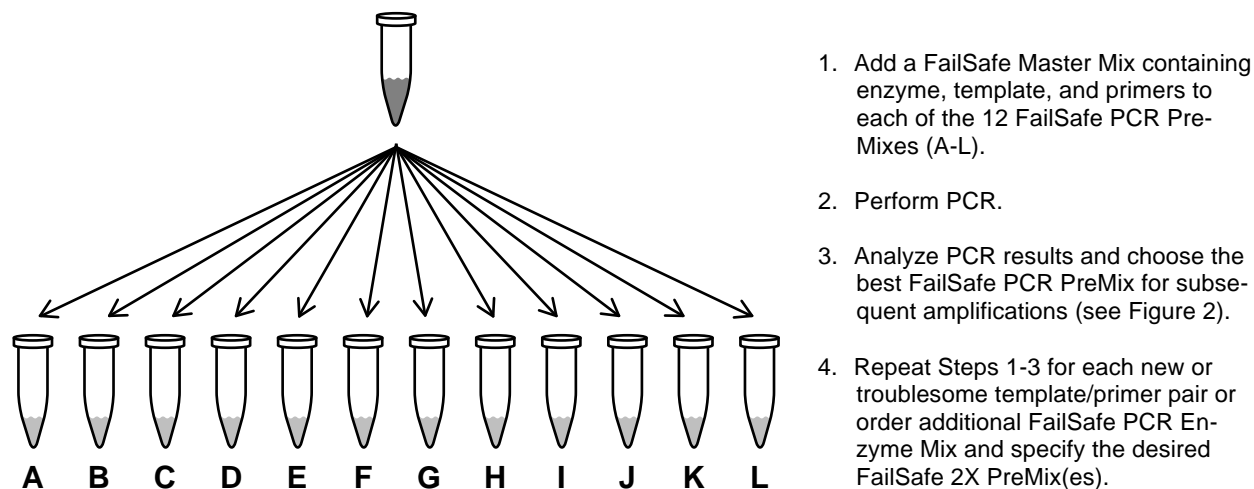
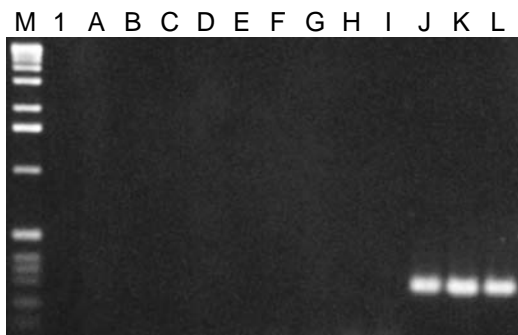
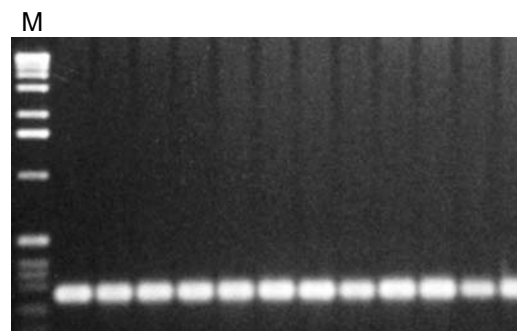


Figure 2. Amplification of a GC-rich template using the FailSafe PCR PreMix Selection Kit.



A 75% GC-rich region of the human *apoE* 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.

Figure 3. Consistent amplification with FailSafe PCR PreMix K.



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

Related Products: The following products are also available:

- TAQXpedite™ PCR System (FAST end-point)
- MasterAmp™ Buccal Swab DNA Extraction Kits
- MasterPure™ Nucleic Acid Purification Kits
- BuccalAmp™ DNA Extraction Kits

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- μ l 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.

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

If amplification ≤ 10 kb:

- 0.5 μ l FailSafe PCR Enzyme Mix (1.25 Units)

If amplification > 10 kb:

- 1.0 μ l FailSafe PCR Enzyme Mix (2.5 Units)
- 25 μ l Total reaction volume

2. On ice, aliquot 25 μ l of each FailSafe PCR 2X PreMix into an individual PCR tube.
3. Add 25 μ l of the FailSafe Master Mix from Step 1 to the PCR tubes and mix.
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 $T_m \geq 65^\circ\text{C}$.
 - 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 - 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 .

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 92-98°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.

(Continued on next page)

- 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 preheated to 92-98°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 1-4°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 5.

FailSafe™ PCR Components

FailSafe PCR PreMix Selection Kit

FailSafe PCR Enzyme Mix	@ 2.5 U/μl	60 U
FailSafe PCR 2X PreMixes	A thru L	each 100 μl
Cat. # FS99060		

Note: Each PreMix volume has been modified to match the Enzyme Mix volume.

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
Cat. # FS99100		

FailSafe PCR Enzyme Mix	@ 2.5 U/μl	250 U
Choice of 2 FailSafe PCR 2X PreMixes		each 2.5 ml
Cat. # FS99250		

FailSafe PCR Enzyme Mix	@ 2.5 U/μl	1,000 U
Choice of 8 FailSafe PCR 2X PreMixes		each 2.5 ml
Cat. # FS9901K		

FailSafe Enzyme Mix Only**

FailSafe PCR Enzyme Mix	@ 2.5 U/μl	100 U
Cat. # FSES1100		40 μl

FailSafe PCR Enzyme Mix	@ 2.5 U/μl	1,000 U
Cat. # FSES101K		400 μl

Individual FailSafe PCR 2X PreMixes

FailSafe PCR 2X PreMix A	Cat. # FSP995A	2.5 ml
FailSafe PCR 2X PreMix B	Cat. # FSP995B	2.5 ml
FailSafe PCR 2X PreMix C	Cat. # FSP995C	2.5 ml
FailSafe PCR 2X PreMix D	Cat. # FSP995D	2.5 ml
FailSafe PCR 2X PreMix E	Cat. # FSP995E	2.5 ml
FailSafe PCR 2X PreMix F	Cat. # FSP995F	2.5 ml
FailSafe PCR 2X PreMix G	Cat. # FSP995G	2.5 ml
FailSafe PCR 2X PreMix H	Cat. # FSP995H	2.5 ml
FailSafe PCR 2X PreMix I	Cat. # FSP995I	2.5 ml
FailSafe PCR 2X PreMix J	Cat. # FSP995J	2.5 ml
FailSafe PCR 2X PreMix K	Cat. # FSP995K	2.5 ml
FailSafe PCR 2X PreMix L	Cat. # FSP995L	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.

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TA Cloning is a registered trademark of Invitrogen, San Diego, California.

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+ Use of betaine in DNA polymerase reactions, including, but not limited to use for PCR or DNA sequencing, is covered by U.S. Patent No. 6,270,962, European Patent No. 0742838, German Patent No. DE4411588C1, and other patents or patent applications in the U.S. and other countries that are either assigned or exclusively licensed to EPICENTRE. Purchase of a product from EPICENTRE that contains betaine and a thermostable DNA polymerase is accompanied by a limited non-exclusive license for the purchaser to use the purchased product solely for life science research, whether the purchaser performs research in a not-for-profit or a for-profit organization. However, if the product does not contain a thermostable DNA polymerase in addition to betaine, all for-profit organizations require a license from EPICENTRE in order to use betaine or a product that contains betaine in DNA polymerase reactions for research applications, and not-for-profit organizations require a license if the product, the research, or the result of the research is transferred to or obtained for or on behalf of a for-profit organization. Licenses are also available to use betaine in DNA polymerase reactions for human or animal diagnostics, screening, or other fields of use. Please contact EPICENTRE for information related to such licenses.

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