Lucigen®

Simplifying Genomics

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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

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, on a gel, 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.[1-3] In addition, betaine also may enhance PCR by protecting DNA polymerases from thermal denaturation.[4] 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.[1,2]

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.

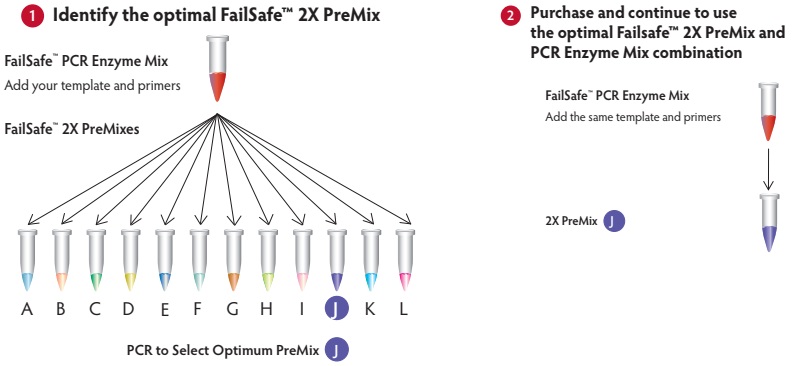

Figure 1. Never fail at PCR again. An overview of the FailSafe™ PCR PreMix Selection Kit and FailSafe™ PCR System workflow.

2. Product Designations and Kit Components

Product	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
FailSafe™ PCR PreMix Selection Kit (all 12 Premixes)	60 Units	FS99060	FailSafe™ PCR Enzyme Mix (2.5 U/µL)	E0030-2.5D1	24 µL
			FailSafe™ PCR 2X PreMix A	SS000148-D1	100 µL
			FailSafe™ PCR 2X PreMix B	SS000149-D1	100 µL
			FailSafe™ PCR 2X PreMix C	SS000150-D1	100 µL
			FailSafe™ PCR 2X PreMix D	SS000151-D1	100 µL
			FailSafe™ PCR 2X PreMix E	SS000152-D1	100 µL
			FailSafe™ PCR 2X PreMix F	SS000153-D1	100 µL
			FailSafe™ PCR 2X PreMix G	SS000154-D1	100 µL
			FailSafe™ PCR 2X PreMix H	SS000155-D1	100 µL
			FailSafe™ PCR 2X PreMix I	SS000156-D1	100 µL
			FailSafe™ PCR 2X PreMix J	SS000157-D1	100 µL
			FailSafe™ PCR 2X PreMix K	SS000158-D1	100 µL
			FailSafe™ PCR 2X PreMix L	SS000159-D1	100 µL
FailSafe™ PCR System with PreMix Choice (any one PreMix)	100 Units	FS99100	FailSafe™ PCR Enzyme Mix (2.5 U/µL)	E0030-2.5D2	40 µL
			1 of FailSafe 2X PreMix (FSP995A-L)	n/a	n/a
FailSafe™ PCR System with PreMix Choice (any two PreMixes)	250 Units	FS99250	FailSafe™ PCR Enzyme Mix (2.5 U/µL)	E0030-2.5D3	100 µL
			2 of FailSafe 2X PreMixes (FSP995A-L)	n/a	n/a
FailSafe™ Enzyme Mix Only	100 Units	FSE51100	FailSafe™ PCR Enzyme Mix (2.5 U/µL)	E0030-2.5D2	40 µL
	1,000 Units	FSE5101K		E0030-2.5D4	400 µL
FailSafe™ PCR 2X PreMix A	2.5 mL	FSP995A	FailSafe™ PCR 2X PreMix A	SS000148-D2	2.5 mL
FailSafe™ PCR 2X PreMix B	2.5 mL	FSP995B	FailSafe™ PCR 2X PreMix B	SS000149-D2	2.5 mL
FailSafe™ PCR 2X PreMix C	2.5 mL	FSP995C	FailSafe™ PCR 2X PreMix C	SS000150-D2	2.5 mL
FailSafe™ PCR 2X PreMix D	2.5 mL	FSP995D	FailSafe™ PCR 2X PreMix D	SS000151-D2	2.5 mL
FailSafe™ PCR 2X PreMix E	2.5 mL	FSP995E	FailSafe™ PCR 2X PreMix E	SS000152-D2	2.5 mL
FailSafe™ PCR 2X PreMix F	2.5 mL	FSP995F	FailSafe™ PCR 2X PreMix F	SS000153-D2	2.5 mL

Product	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
FailSafe™ PCR 2X PreMix G	2.5 mL	FSP995G	FailSafe™ PCR 2X PreMix G	SS000154-D2	2.5 mL
FailSafe™ PCR 2X PreMix H	2.5 mL	FSP995H	FailSafe™ PCR 2X PreMix H	SS000155-D2	2.5 mL
FailSafe™ PCR 2X PreMix I	2.5 mL	FSP995I	FailSafe™ PCR 2X PreMix I	SS000156-D2	2.5 mL
FailSafe™ PCR 2X PreMix J	2.5 mL	FSP995J	FailSafe™ PCR 2X PreMix J	SS000157-D2	2.5 mL
FailSafe™ PCR 2X PreMix K	2.5 mL	FSP995K	FailSafe™ PCR 2X PreMix K	SS000158-D2	2.5 mL
FailSafe™ PCR 2X PreMix L	2.5 mL	FSP995L	FailSafe™ PCR 2X PreMix L	SS000159-D2	2.5 mL

3. 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.

The FailSafe™ PCR PreMix Selection Kit is sufficient for 48 reactions or four, 12-reaction optimization runs.

Note: *Lucigen 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.*

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.

4. 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.[5,6]

   Note: *Depending on the complexity of the genomic DNA sample, up to 100 ng can be used as template in a 50 µL reaction.*

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. The use of a primer design program, such as IDT's

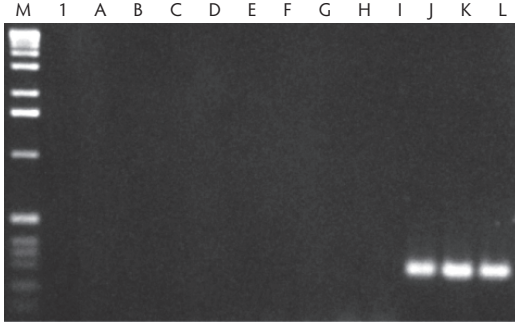

Figure 2. Amplification of a GC-rich template using the FailSafe™ PCR PreMix Selection Kit. A 75% GC-rich region of the human *Apo*E gene was amplified. M, molecular marker; Lane 1, standard buffer conditions using MasterAmp *Taq* DNA polymerase; Lanes A-L, amplification with each of the 12 FailSafe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

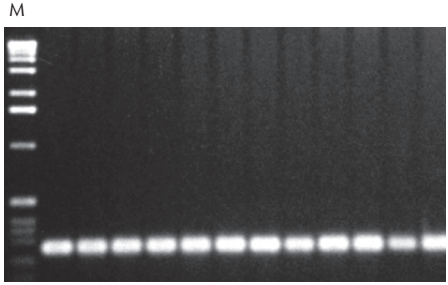

Figure 3. Consistent amplification with FailSafe™ PCR PreMix K. Twelve repeat amplification reactions of the *Apo*E gene from Fig. 2 were performed using FailSafe PCR 2X PreMix K. M, molecular marker.

Primerquest or Primer 3, may simplify primer design to eliminate possible primer-dimers or cross-reactivity.

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.[7] 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.[5] Additional variations such as use of a hot start[8] or touchdown/stepdown PCR[9,10] can dramatically improve specificity and yield (see page 6 Troubleshooting Amplification Reactions).
5. **Cloning PCR Products:** Three PCR product types are produced in each reaction: (i) Non-template As on the 3′ end; (ii) one "A" and one "blunt" end on either the 5′ or 3′ end; and (ii) blunt ends on either the 5′ or 3′ ends of the PCR products.

   Hence, an efficient number of clones can be obtained with either blunt-end cloning or TA Cloning® strategies.
6. **Scalability:** It is possible to scale down FailSafe PCR projects by proportionally decreasing each reagent (primers, enzyme, and Premix).

5. 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.

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)
y	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.
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.

6. Troubleshooting Amplification Reactions

Little or no amplification detected

1) **Lower annealing temperature.** Lower the annealing temperature in 2°C increments.
2) **Perform hot start.**[8] 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.**[5,9,10] 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 µM 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. Addition of ~3 cycles increases the product mass by two-fold.

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).[5,10] 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).[5,10] 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.**[8] 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.**[5,9,10] 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. See Section 5 for information on the use of primer design software.

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.

7. References

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