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ProductInformation

fasL PCR Primers Set

Product No. **F 8300**
Store at –20 ºC

Product Description

Apoptosis, or programmed cell death (PCD), is a process essential for safeguarding of the genomic DNA-integrity, of cell in the developmental biology of multicellular organism and in tissue homeostasis of the adult animal. Among the molecular mediators triggering the apoptotic cascade there is the **Fas receptor** and its ligand **(Fas Ligand, FasL)**. Fas (CD95, APO-1) is a surface (trans-membrane) receptor that is a member of the TNF receptor/nerve growth factor receptor family. Fas receptor mediates apoptosis in a wide variety of cell types.[1-2]

In recent years, the Fas system has been implicated as a key factor in the homeostatic regulation of the immune response being involved in the activation-induced cell death (AICD). In addition induction of the Fas receptor on target cells by autoreactive T cells has been demonstrated to play a central role in tissue destruction in some autoimmune diseases, such as Hashimoto's thyroiditis in humans and autoimmune diabetes in non-obese diabetic mice.[3-6]

The FasL-PCR Primer Set contains sense and antisense primers for the amplification of FasL gene. It is directed for the detection of human, rat and mouse cDNA levels (represents mRNA expression) of FasL gene. No amplification of the genomic DNA has been observed

The size of the amplified product resulting from the use of the primers set is 463, 457and 454 bp for Human, Mouse and Rat, respectively.

Equipment and Reagents Required but Not Provided (Sigma product numbers have been given where appropriate)

- Thermal cycler
- Taq DNA polymerase, Product No. D4545 or equivalent
- Deoxynucleotide mix, 10 mM, Product No. D7295 or equivalent
- Agarose
- Ethidium bromide, 500 µg/ml, Product No. E1385
- PCR 100 bp low ladder, Product No. P1473
- Gel loading solutions, Product No. G2526 or G7654
- PCR grade water, Product No. W1754
- Mineral oil, Product No. M8662
- PCR microtubes, Product No. Z37,487-3 or Z37,496-2

Storage

Store the vial at –20 °C.

Preparation Instructions

The fasL PCR Primers Set contains 1 nmoles of each primer (sense and antisense). Centrifuge the tube briefly in order to collect the tube contents. For the following procedure, resuspend the primers set in 100 µl deionized water to a final concentration of 10 pmole/µl. Mix until the solution is homogenous. Once suspended, store the solution at -20 °C. To avoid repeated freeze-thaw cycles, aliquot the primer solution for long-term storage.

Procedure

Note: Use aseptic techniques and use aerosol barrier tips while performing PCR experiments.

1. Thaw the fasL PCR Primers Set on ice, being sure that the solution is homogenous.
2. Add the following reagents to a PCR microcentrifuge tube in the following order:

	Amount for 50 µl single PCR reaction	Final concentration in the PCR reaction
Water	To 50 µl	----
10X PCR Buffer	5 µl	1X
2 mM dNTP solution	5 µl	0.2 mM of each dNTP
25 mM $MgCl_2$* solution	1-1.5 µl	0.5-0.8 mM
fasL PCR Primers Set, 10 pmole/µl	2 µl	0.4 µM
CDNA**	2 µl	~30 ng
Taq DNA Polymerase, 5 units/µl	1 µl	0.1 units/µl
Total volume	50 µl	-

* When using the bak PCR Primers Set for the first time, you may set two additional reaction tubes with a higher and a lower $MgCl_2$ concentrations (see Note at the end of this section).

** Optimize this parameter with your own cDNA.

3. Mix gently by finger tapping and centrifuge briefly to collect the mixture in the bottom of the tube. Overlay the reaction mixture with 2 drops (~30 µl) of mineral oil to cover the surface of the reaction mixture if not using a thermal cycler with a heated lid.

Place the tube in the thermal cycler when the thermal cycler reaches 95°C, and run the following PCR program.

94°C for 2 min

94°C for 1 min
55°C for 1 min
72°C for 1.5 min
] x 35-40 cycles

72°C for 7 min

The amplified DNA can be evaluated by agarose gel electrophoresis.

Note: Using different thermal cyclers:
For a better detection of the amplified product you may increase the number of amplification cycles. In case you do not see differences in the amount of the amplified DNA fragments, decrease the number of cycles to verify your results.

In rare cases, some of the parameters should be optimized for the specific thermal cycler or cDNA samples. The most frequently adjusted factors are $MgCl_2$ concentration and annealing temperature. To minimize further requirements for optimization, the first time you use the primers you may prepare three different reactions using $MgCl_2$ at a concentration of 0.5-3 mM (e.g., 0.5-0.8 mM, 1.5 mM and 3 mM).

Troubleshooting Guide

Problem	**Cause**	**Solution**
No PCR products	A PCR component may be missing or degraded.	Try to isolate the problematic reagent by replacing it with a fresh one. A checklist is also recommended when assembling reactions.
	cDNA or $MgCl_2$ concentration is not optimal.	Optimize the cDNA and $MgCl_2$ concentrations.
High background, smearing or nonspecific bands		Increase the annealing temperature or decrease the $MgCl_2$ concentration. Another solution for avoiding high background is to decrease the amount of cDNA template used for amplification.
Amplified products are not the correct size	Contamination with other DNA	Use sterile techniques while performing PCR experiments.
	CDNA quality is not sufficient	Use a different cDNA preparation.
	Non-optimal PCR conditions	Optimize PCR conditions especially cDNA and $MgCl_2$ concentrations and annealing temperature.
Poor resolution of products in agarose gel		Use 2% agarose gel and increase run time.

References

1. Oehm, A., et al., .J. Biol. Chem., **267**, 10709 (1992).
2. Itoh, N., et al., Cell, **66**, 233 (1991).
3. Nagata, S., and Golstein, P., Science, **267**, 1449 (1995).
4. Lynch, D.H., et al., Immunol. Today, **16**, 569 (1995).
5. Giordano, C., et al., Science, **275**, 960 (1997).
6. Chervonsky, A.V., et al., Cell, **89**, 17 (1997).

Related Products

- mRNA Isolation Kits, MRI-1 (micro kit) and MRI-2 (standard kit)
- Enhanced Avian First Strand Synthesis Kit, STR-1

[†]The PCR process is covered by patents owned by Hoffman-LaRoche, Inc. Purchase of this product does not convey a license under these patents.

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