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P R O D U C T I N S E R T

SSP B27 Liquid Bulk Primer Set

Catalog #s BSSPB2773, BSSPB2781, BSSPB27C

For research use only. Not for use in diagnostic procedures.

STORE REAGENTS AT TEMPERATURE INDICATED ON PACKAGE. USE BEFORE EXPIRATION DATE.

Warning: *Use only the D-mix packaged with this SSP B27 Liquid Bulk Primer Set.*

DIRECTIONS FOR USE:

Formulation

Use the following formulation to prepare the reagents for your B27 test:

1. Use 2µl of primer set per PCR reaction.
2. Use 1µl of the test sample and 7 µl D-mix per reaction.
3. Use .05 µl at 5 units/µl of recombinant Taq polymerase per reaction.
4. Perform standard PCR with the Micro SSP™ PCR program (OLI-1) as specified below.

SUMMARY AND EXPLANATION

Historically, the established method for the determination of HLA antigens has been the lymphocytotoxicity test.[1] With the advent of PCR technologies, DNA-based tissue typing techniques have become routine in the laboratory. For most DNA-based methodologies, the PCR process is used only as an amplification step to acquire the needed target DNA. The HLA typing process then requires a post-amplification step to discriminate between the different alleles (e.g., RFLP, SSOP, reverse dot blot). Unlike other PCR-based methods, the SSP methodology employed by One Lambda Inc. discriminates between the different alleles during the PCR process[2]. This shortens the post-amplification processing time to a simple gel electrophoresis detection step. In contrast to the lymphocytotoxicity reaction scale (1 = negative to 8 = positive), SSP test results are either positive or negative. This eliminates the need for complicated interpretation of results. In addition, single nucleotide changes can be discriminatory in PCR-SSP, while cross-reacting groups (CREGs) provide major challenges to serological typing.[3] Finally, due to the synthetic nature of DNA typing reagents (e.g., oligonucleotide primers) stability and lot to lot variance have been greatly improved.

COMPONENTS (50 tests)

1. 100 µl primer (2µl per test)
2. 1000 µl D-mix (7 µl per test)

Note: *The volumes provided are slightly more than the amount required for testing. This is to account for inadvertent losses which may result from pipetting.*

PCR PROGRAM

The following program is designed for use only on the Perkin-Elmer 9600 or 9700 thermocyclers. If you have a different make and/or model thermocycler, you may need to adapt the programming to your machine. Program your thermocycler before starting the "Directions for Use."

Micro SSP™ PCR Program (OLI-1):

# of Cycles	Step	Temp (°C)	Time (sec)
1	1	96	130
	2	63	60
9	1	96	10
	2	63	60
20	1	96	10
	2	59	50
	3	72	30
End	1	4	---

PRECAUTIONS AND LIMITATIONS

1. For research use only.
2. Limit refreezing of primers no more than twice.
3. Pipettes used for **Post**-PCR manipulations should **not** be used for **Pre**-PCR manipulations.

PRECAUTIONS AND LIMITATIONS (continued)

4. If salts have precipitated out of solution in the D-mix aliquots during shipping or storage, re-dissolve by extended vortexing at room temperature.
5. D-mix aliquots, upon thawing at room temperature (20 - 25°C), should be pink to light purple in color. Any D-mix aliquot without the specified coloration should be considered unusable. Aliquot D-mix into convenient volumes. Do not refreeze more than twice.
6. Biohazard Warning: The ethidium bromide used for staining of DNA is a potential carcinogen. Always wear gloves when handling stained gels.
7. Biohazard Warning: All blood products should be treated as potentially infectious.
8. Caution: Wear UV-blocking eye protection, and do not view UV light source directly when viewing or photographing gels.

PRIMER RECOGNITION SITES

Alleles	5' Recognition Site	3' Recognition Site	Approx. Size Base Pairs
B2701-14; B7301	48T48	68-E69	100
B2701-14; B8101	16-V17	(68RE69 & 75-76)	325

REFERENCES

1. Terasaki, P.I., Bernoco, F., Park, M.S., Ozturk, G., and Iwaki, Y. Microdroplet testing for HLA-A, -B, -C and –D antigens. American Journal of Clinical Pathology 69:013-120, 1978.
2. Newton, C.R.., Graham, A. Heptinstall, E., Powell, S.J., Summers, C., Kalsheker, N., Smith J.C., and Markham, A.F. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Research 17: 2503-2516, 1989.
3. Slater, R.D. and Parham, P. Mutually exclusive public epitopes of HLA-A, B, C molecules. Human Immunology 26: 85-89, 1989.

TRADEMARKS USED IN THIS DOCUMENT/ PRODUCT

Micro SSP™	One Lambda Inc.
Gene-Amp®	Hoffmann-LaRoche, Inc
ARMS™	Zeneca Limited

PATENTS USED IN THIS DOCUMENT

Nothing in this publication should be construed as an authorization or an implicit license to practice PCR under any patents held by Hoffmann-LaRoche, Inc.

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