Apoptosis, or programmed cell death (PCD), is a process essential for safeguarding the integrity of genomic DNA in the cell in the developmental biology of multi-cellular organism and in tissue homeostasis of the adult animal. The proper regulation of apoptosis is as important as the control of cell proliferation and the regulation of the cell cycle. Diseases associated with failed apoptosis or undesirable cell survival include cancer, autoimmune disorders and viral infections. In recent years, several genes have been linked to apoptosis. The bcl-2 family of genes regulates PCD either positively or negatively. Bcl-2 and members of its family have been found to block apoptotic cell death. Bcl-2 protein heterodimerizes with Bax (Bcl-2 Associated X protein), which is a potent mediator of programmed cell death. The Bcl-2/Bax ratio appears to determine whether some cells live or die.1-4 The Apoptosis PCR bax/bcl-2 Multiplex Primer Set is designed for multiplex RT-PCR† detection of Human, Rat and Mouse cDNA levels (represents mRNA expression) of bcl-2α and baxα apoptotic genes, and a control housekeeping gene, GAPDH. No amplification of the genomic DNA has been observed for all three genes.

The kit contains PCR primers sets for bcl-2, bax and GAPDH genes and a control cDNA. Each primer set contains sense and antisense primers for the amplification of one of the genes.

The sizes of the amplified products resulting from the use of these kit primers are 487 bp for bax-α, 349 bp for GAPDH and 127 bp for bcl-2α.

Reagents Provided
Sufficient for fifty 50 µl PCR reactions

- Control cDNA (mouse spleen), 1 vial
  Product No. C9227

- bax PCR primers set, Product No. B8304 1 vial
  Reverse primer sequence (3' antisense): 5'-CAT CTT CCA GAT GGT GA-3'
  Forward primer sequence (5' sense): 5'-GTT TCA TCC AGG ATC GAG CAG-3'

- bcl-2 PCR primers set, Product No. B9179 1 vial
  Reverse primer sequence (3' antisense): 5'-GAG ACA GCC AGG AGA AAT CA-3'
  Forward primer sequence (5' sense): 5'-CCT GTG GAT GAC TGA GTA CC-3'

- GAPDH PCR primers set, 1 vial
  Product No. P7732
  Reverse primer sequence (3' antisense): 5'-YGC CTG CTT CAC CTT C-3'
  Forward primer sequence (5' sense): 5'-TGC MTC CTG CAC CAC CAA CT-3'
  where M = A or C
  Y = T or C

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 kit at \(-20^\circ\text{C}\).

Preparation Instructions
The provided kit components are dried and should be resuspended in water. Once suspended, components should be stored at \(-20^\circ\text{C}\). To avoid repeated freeze-thaw cycles, aliquot the primer solutions for long-term storage.

1. Control cDNA (mouse spleen): Centrifuge the tube briefly in order to collect the tube contents. Resuspend the cDNA in 10 \(\mu\text{l}\) deionized water. Mix gently. Store at \(-20^\circ\text{C}\).

2. bax, bcl-2 and GAPDH PCR primers sets: The bax and bcl-2 primers sets contain 1 nmoles of each primer (sense and antisense). The GAPDH primers set contains 0.5 nmoles of each primer (sense and antisense). Centrifuge each tube briefly in order to collect the tube contents. Resuspend each primer set in 100 \(\mu\text{l}\) deionized water to a final concentration of 10 pmole/\(\mu\text{l}\) (for bax and bcl-2) and 5 pmole/\(\mu\text{l}\) (for GAPDH). Mix until solutions are homogenous.

3. 2 mM dNTP solution. Dilute the 10 mM Deoxynucleotide Mix (Product No. D7295) 1:4 with PCR grade water to give a 2 mM dNTP solution (e.g. 1 part D7295 plus 4 parts PCR grade water).

Procedure

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

1. Thaw all kit components on ice, being sure that all solutions are homogenous.

2. Add the following reagents to a PCR microcentrifuge tube in the following order:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount for 50 (\mu\text{l}) single PCR reaction</th>
<th>Final concentration in the PCR reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>28 (\mu\text{l})</td>
<td>----</td>
</tr>
<tr>
<td>10X PCR Buffer</td>
<td>5 (\mu\text{l})</td>
<td>1X</td>
</tr>
<tr>
<td>2 mM dNTP solution</td>
<td>5 (\mu\text{l})</td>
<td>0.2 mM of each dNTP</td>
</tr>
<tr>
<td>25 mM MgCl(_2) solution</td>
<td>3 (\mu\text{l})</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>bax PCR Primers set, 10 pmole/(\mu\text{l})</td>
<td>2 (\mu\text{l})</td>
<td>0.4 (\mu\text{M})</td>
</tr>
<tr>
<td>bcl-2 PCR Primers set, 10 pmole/(\mu\text{l})</td>
<td>2 (\mu\text{l})</td>
<td>0.4 (\mu\text{M})</td>
</tr>
<tr>
<td>GAPDH PCR Primers set, 5 pmole/(\mu\text{l})</td>
<td>2 (\mu\text{l})</td>
<td>0.2 (\mu\text{M})</td>
</tr>
<tr>
<td>cDNA*</td>
<td>2 (\mu\text{l})</td>
<td>~30 ng</td>
</tr>
<tr>
<td>Taq DNA Polymerase, 5 units/(\mu\text{l})</td>
<td>1 (\mu\text{l})</td>
<td>0.1 units/(\mu\text{l})</td>
</tr>
<tr>
<td>Total volume</td>
<td>50 (\mu\text{l})</td>
<td>-</td>
</tr>
</tbody>
</table>

- The amount of cDNA required for the amplification reaction varies between cell types and cDNA synthesis and purification systems. The amount of cDNA indicated in the table refers to the control cDNA provided with the kit. If your sample DNA to be amplified is at a different volume, adjust the water added accordingly to give a final volume of 50 \(\mu\text{l}\).

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 \(\mu\text{l}\)) 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\(^\circ\text{C}\), and run the following PCR program.

\[
\begin{align*}
95^\circ\text{C} & \text{ for } 2 \text{ min} \\
94^\circ\text{C} & \text{ for } 45 \text{ sec} \\
53^\circ\text{C} & \text{ for } 45 \text{ sec} \\
72^\circ\text{C} & \text{ for } 1.5 \text{ min} \\
72^\circ\text{C} & \text{ for } 7 \text{ min}
\end{align*}
\times 30 \text{ cycles}
\]
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 MgCl₂ concentration and annealing temperature. You may prepare three different reactions using MgCl₂ at a concentration of 0.5-3 mM (e.g., 0.5-0.8 mM, 1.5 mM and 3 mM). Optimize the MgCl₂ and/or the annealing temperature on your instrument using the positive control cDNA provided before using your own cDNA.

The bax, bcl-2, and GAPDH primers set can be amplified separately using the same procedure. For individual amplifications, replace the omitted primers with water. The total reaction volume is 50 µl.

### Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No PCR products</td>
<td>A PCR component may be missing or degraded</td>
<td>A positive control should be run to insure components are functioning. A checklist is also recommended when assembling reactions.</td>
</tr>
<tr>
<td></td>
<td>cDNA or MgCl₂ concentration is not optimal</td>
<td>Optimize the cDNA and MgCl₂ concentrations.</td>
</tr>
<tr>
<td>One of the genes is not amplified</td>
<td>Reaction conditions (cDNA and other components or the enzyme) are not optimal for multiplex PCR</td>
<td>Amplify each primers set separately. Afterwards, try to amplify two of them and then all three. In rare cases a change by the same factor of the concentration of all three primers in the reaction mixture may improve amplification of problematical cDNA.</td>
</tr>
<tr>
<td>The GAPDH band is too thin or too thick compared to other bands</td>
<td>The copy number of GAPDH cDNA in your reaction mixture is very high (for thick band) or low (for thin band)</td>
<td>Optimize the GAPDH primers set concentration to your cDNA.</td>
</tr>
<tr>
<td>High background, smearing or nonspecific bands</td>
<td></td>
<td>Increase the annealing temperature or decrease the MgCl₂ concentration. Another solution for avoiding high background is to decrease the amount of cDNA template used for amplification.</td>
</tr>
<tr>
<td>Amplified products are not the correct size</td>
<td>Contamination with other DNA</td>
<td>Use sterile techniques while performing PCR experiments.</td>
</tr>
<tr>
<td></td>
<td>cDNA quality is not sufficient</td>
<td>Use a different cDNA preparation.</td>
</tr>
<tr>
<td></td>
<td>Non-optimal PCR conditions</td>
<td>Optimize PCR conditions especially cDNA and MgCl₂ concentrations and annealing temperature.</td>
</tr>
<tr>
<td>Poor resolution of products in agarose gel</td>
<td></td>
<td>Use 2% agarose gel and increase run time.</td>
</tr>
</tbody>
</table>
Related Products

- mRNA Standard Isolation Kit, Product No. MRI-2

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

References