human Survivin RT-PCR Primers

Catalog Number: RDP-204-025
Pack Size: 25 tests
Lot Number:
Expiration Date:

Specifications and Use

Description

- Human Survivin specific RT-PCR* primers (reverse transcription followed by polymerase chain reaction) for analysis of mRNA expression.

Components

- **Gene-specific RT Primer -** Lyophilized. Each vial contains 50 pmoles. Adjust to a final concentration of 2 pmoles/µL by resuspending the primer in 25 µL nuclease-free water.
- **PCR Primer Pair** - Lyophilized. Each vial contains 375 pmoles of each primer. Adjust to a final concentration of 7.5 pmoles/µL by resuspending Primer Pair in either 50 µL autoclaved deionized water or 0.1X TE buffer (1 mM Tris HCl, pH 8.0 @ 25° C; 0.1 mM EDTA, pH 8.0 @ 25° C).
- **Positive Control 33** - Lyophilized. Each vial contains 150 ng synthetic double-stranded DNA. Resuspend in 30 µL autoclaved deionized water or TE buffer. To prevent contaminating samples and reagents, it is strongly recommended that the Positive Control is resuspended in a separate location from where PCR reactions are set up. Use a different pipette than one used for PCR set up. The Positive Control is not intended for quantitative purposes.

Sequence

- GenBank® Accession Number: NM_001168

Product Sizes

- **cDNA**: 199 base pairs (bp). Use gene-specific primer for reverse transcription.
- **Genomic DNA**: 451 bp product predicted and observed.
- **Positive Control 33**: 320 bp
- **Alternate splice variants**: Exist outside region amplified.

Storage

- The gene-specific and PCR primers resuspended in water or TE buffer are stable for up to one year at ≤ -20° C in a non-frost free freezer. Aliquot in single use portions. **Do not use past the expiration date above. Avoid repeated freeze-thaw cycles.**

We recommend the following protocols for R&D Systems' RT-PCR Primers.

Reverse Transcription Reaction:

1. Thaw all reagents completely on ice. All reactions should be assembled on ice.
2. Resuspend the RT primer according to the instructions in the "Specifications and Use" section.
3. Pipet the following into a nuclease-free tube:
   1 to 5 µg of total RNA (up to 11 µL)
   1 µL Gene Specific RT primer (2 pmoles/µL)
   x µL Nuclease-free dH_2O for a final volume of 12 µL
4. Mix and incubate at 70° C for 10 minutes. Place tube on ice immediately.
5. Briefly centrifuge the tube, then add the following to each tube:
   4 µL 5X Reverse Transcription Buffer
   2 µL 0.1M DTT
   1 µL 10 mM dNTPs
6. Mix and incubate at room temperature (~25° C) for 10 minutes.
7. Incubate at 42° C for 2 minutes.
8. Add 1 µL RNase H⁻ Reverse Transcriptase (200 units/µL). Mix by pipetting.
9. Incubate at 42° C for 50 minutes.
10. Incubate at 70° C for 15 minutes.
11. Dilute reactions 5-fold by adding 80 µL of nuclease-free dH_2O.
12. Store at ≤ -20° C in a manual defrost freezer.

For the PCR Reaction, please turn to page 2.

Technical Hints

To minimize the risk of amplicon contamination of the Primer Pair and other PCR reagents, the following is recommended:

PCR reactions should be set up in an area separate from where PCR products are analyzed.
Pipettes and tube racks should be specifically designated for PCR.
Use aerosol barrier pipette tips.

PCR Reaction:

1. Thaw all reagents completely on ice. All reactions should be assembled on ice.
2. Resuspend the PCR Primer Pair according to the instructions in the "Specifcations and Use" section. (Do not resuspend the Positive Control at this time.)
3. Determine the number of PCR reactions. Multiply the volumes listed below for each reagent by the number of reactions. Prepare a PCR master mix on ice.
   - 36.5 µL autoclaved deionized water
   - 5 µL 10X Taq buffer (with 15 mM $MgCl_2$)
   - 5 µL 10X dNTP mix (10X = 2 mM each dNTP)
   - 2 µL Primers (7.5 µM each primer)
   - 0.5 µL Taq DNA Polymerase (5 units/µL)
4. Prepare the negative control reaction tube.
   a) Pipet 1 µL of autoclaved deionized water into a pre-labeled negative control tube.
   b) Add 49 µL of the master mix prepared above.
   c) Briefly spin tube and add 30 µL of mineral oil to prevent evaporation.
   d) Close the reaction tube and place on ice.
5. Prepare the cDNA sample reaction tubes.
   a) Pipet 1 µL of cDNA sample into a pre-labeled PCR reaction tube.
   b) Add 49 µL of the master mix prepared above.
   c) Briefly spin tube and add 30 µL of mineral oil to prevent evaporation.
   d) Close the reaction tube and place on ice.
6. Resuspend the Positive Control in 30 µL of autoclaved deionized water. Centrifuge the Positive Control tube briefly. This should be done in a separate location from where PCR reactions are set up. Use different pipettes than those used for PCR set up.
   a) Pipet 1 µL of Positive Control into the pre-labeled Positive Control reaction tube.
   b) Add 49 µL of the master mix prepared above.
   c) Briefly spin tube and add 30 µL of mineral oil to prevent evaporation.
   d) Close the reaction tube and place on ice.
7. Place all tubes in thermal cycler and perform the following program. (Note: The recommended annealing temperature for is 55° C).

   94 °C for 4 minutes

   94 °C for 45 seconds
   55 °C for 45 seconds
   72 °C for 45 seconds
   } x 30-35 cycles

   72 °C for 10 minutes

Analysis of Results: The PCR products can be analyzed by agarose gel electrophoresis. For predicted sizes of PCR products, refer to the "Specifications and Use" section.

Troubleshooting

Problem	Suggestions
No PCR products obtained	• Operator error may have occurred during reaction assembly. Run positive control reaction. • Unsuccessful cDNA synthesis. Repeat PCR reaction with primers for a housekeeping gene as a control to show that RT was successful. • RNA may be degraded. Check the integrity of RNA by gel electrophoresis. A good quality RNA preparation should show the 28S and 18S ribosomal RNAs in an approximate 2:1 ratio. If RNA appears degraded, repeat RNA isolation. • RNA secondary structure may be inhibiting cDNA synthesis. • The target RNA concentration may be too low. Increase the amount of cDNA template used in PCR reaction or the number of cycles used to amplify the cDNA.
Smearing or bands of unpredicted size	• Non-specific priming may have occurred.