Human XCR1 PCR Primer Pair™

Catalog Number: RDP-245
Pack Size: 25 tests
Lot Number:
Expiration Date:

Specifications and Use

Description

- Human XCR1 (CCXCR1/GPR5) specific PCR* primers for RT-PCR (reverse transcription followed by polymerase chain reaction) analysis of mRNA expression.

Components

- **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 40** - Lyophilized. Each vial contains 150 ng of 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 be resuspended in a separate location from where PCR reactions are set up. Use a different pipette than the one used for PCR set up. The Positive Control is not intended for quantitative purposes.

Sequence

- GenBank® Accession Number: NM_005283

Product Sizes

- **cDNA**: 512 base pairs (bp)
- **Genomic DNA**: 512 bp predicted and observed. Treatment of RNA with RNase-free DNase prior to RT is recommended to remove contaminating genomic DNA.
- **Positive Control 40**: 350 bp
- **Alternate splice variants**: None reported

Storage

- The lyophilized Primer Pair is stable for greater than one year at -20° C. The Primer Pair resuspended in water or TE buffer is 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.**

Technical Hints

- Thaw all reagents completely on ice before use.
- Use either random primers or oligo (dT) for reverse transcription.
- All PCR reactions should be assembled on ice.
- The recommended annealing temperature is 55° C.
- To minimize the risk of amplicon contamination of the Primer Pair and other PCR reagents, the following is recommended:
  1. PCR reactions should be set up in an area separate from where PCR products are analyzed.
  2. Pipettes and tube racks should be specifically designated for PCR.
  3. Use aerosol barrier pipette tips.
- Follow the steps below to determine the number of PCR reactions and to calculate the amount of PCR master mix necessary.
  1. Determine the number of cDNA samples that will be analyzed.
  2. Add 2 to the number of cDNA samples to account for a negative and a positive control.
  3. Multiply the number determined in step 2 by 1.1 to account for pipeting error. This is the number of reactions for which the master mix should be made.

  For example, the master mix for 3 cDNA samples should be:
  1. 3 cDNA samples will be analyzed
  2. Plus 2 for negative and positive controls = 5
  3. 5 x 1.1 for pipeting error = 5.5

- Primer Pairs are not validated for use in kinetic RT-PCR.
- Dilute RT reaction five-fold before using in the PCR reaction.

Genbank is a registered trademark of the United States Department of Health and Human Services.

*PCR is covered by US Patent Nos. 4683195 and 4683202 assigned to Hoffmann-La Roche.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

R&D Systems, Inc.
1-800-343-7475

PCR Guide

We recommend the following protocol for R&D Systems' PCR Primer Pairs™.

Thaw all reagents completely on ice before use.

1. Resuspend the Primer Pair in 50 µL of autoclaved deionized water or 0.1X TE buffer for a final concentration of 7.5 µM each primer. (Do not resuspend the Positive Control at this time.)
2. Determine the number of PCR reactions (see Technical Hints for setting up a PCR master mix). Multiply the volumes listed below of 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)
3. 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.
4. 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.
5. Resuspend the Positive Control in 30 µL of autoclaved deionized water or TE buffer. 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.
6. Place all tubes in thermal cycler and perform the following program:
   94° C for 4 minutes

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

   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. This can often be overcome by using random primers. • 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.