Product Specification

Primer Pair for Gene Specific Fragment Amplification

RhD & RhCcEe Genemer™

Shipped at ambient temperature. Store at -20°C
For research use only. **Not for use in diagnostic procedures for clinical purposes.**

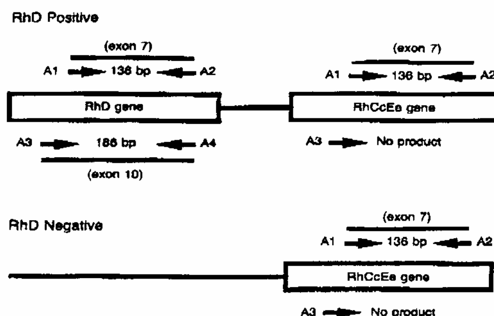
	Product	Catalog Number	Unit Size
❑	RhD Genemer™ RhD gene specific amplification primer pair	40-2002-10	10 nmole
❑	RhCcEe Genemer™ RhCcEe gene specific amplification primer pair	40-2003-10	10 nmole

Background

The Rh based blood grouping is termed positive or negative based on the presence or absence of the D antigen. Rh alloimmunization in Rh negative pregnant women is of concern because of the potential for the fetus to develop hemolytic disease of newborns and autoimmune diseases. Anemia leading to hydrops, perinatal death, or both occurs in 25% of fetuses sensitized to the RhD antigen in the absence of optimal management. In utero diagnosis and treatment considerably improves the condition with survival rates greater than 75% in severely affected fetuses. However these invasive therapies may be unnecessary in some cases if the fetal Rh status were known prenatally.

A method of determining fetal Rh status early in pregnancy is now possible by DNA analysis of amniotic cells (1). The Rh blood group locus consists of two related structural genes, *D* and *CcEe.* These highly homologous genes which share greater than 96% identity in their coding region have been cloned and the molecular basis of the Rh blood group established (2). The RhD-positive and RhD-negative polymorphism is associated with the presence or the absence of the *D* gene (there is no 'd' gene). The C/c and E/e antigens are encoded by a unique gene. The E/e associated nucleotide polymorphism results in one amino acid substitution at position 226 (proline to alanine), whereas the C/c antigenic polymorphism consists of six nucleotide substitutions leading to four amino acid changes at position 16 (Cys16Trp), 60 (Ile60Leu), 68 (Ser68Asn) and 103 (Ser103Pro).

DNA analysis for Rh genotype loci specifically amplifies DNA fragments for the RhD and RhCcEe gene. Due to the high sensitivity and specificity of the test at the DNA level, occasionally the results may not match those obtained by serologic methods. The test will type individuals as RhD positive who are D^u low grade status serologically (3-5), this is due to the absence of the gene product at the protein level due to partial deletions. The total error rate should be less than 1%.



Material Supplied

One tube supplied containing the lyophilized primer pair. Please refer to item number on the top of this sheet (RhD or RhCeEe Genemer™). Each tube contains 10 nmoles of the primers. The quantity supplied is sufficient for 400 regular 50µl PCR reaction.

Results and Interpretation

Genemer™ specific for RhD gene exon 10 (Catalog No. 40-2002-10) will amplify a 186 bp fragment specific and Genemer™ specific for RhCcEe gene exon 7 (Catalog No. 40-2003-10) will amplify a 136 bp fragment as shown in the figure below. Multiplex can be performed.

Normal PCR amplified fragment size		
Genemer™	RhD Genotype	RhCcEe Genotype
RhCeEe	136 bp	136 bp
RhD	186 bp	None
Multiplex RhCeEe & RhD	136 &186 bp	Only 136 bp

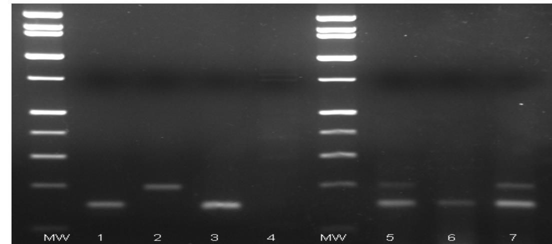


Figure. MW lanes are molecular weight markers. Lanes 1,2, 5 &7 represent PCR from a Rh D positive DNA and Lanes 3,4 & 6 represent PCR from Rh D negative DNA. Lanes 1 & 3 PCR product of RhCcEe Genemer™ amplification, lanes 2 & 4 from RhD Genemer™ amplification. Lane 5, 6 & 7 represent multiplex amplification of RhCcEe and RhD.

References

1. Bennet, P.R., et al. (1993) Prenatal determination of fetal RhD type by DNA amplification. NEJM 329:607-610.
2. Mouro, I., et al. (1993) Molecular genetic basis of the human Rhesus blood group system. Nature Genetics 5:62-65.
3. Simsek, S., Bleeker, P.M., Borne, A.. E.G. (1994) Prenatal determination of fetal RhD type. NEJM 330:795.
4. Bennet, P., Warwick,R. and Carton, J-P. (1994) Prenatal determination of fetal RhD type. NEJM 330:795-796.
Westhoff,C.M. and Wylie, D.E. (1994) Identification of a new RhD-specific mRNA from K562 cells. Blood 84:3098-3100

*The polymerase chain reaction (PCR chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents.

All Gene Link products are for research use only.

140 Old Saw Mill River Road Hawthorne, NY 10532
Tel: 914.769.1192 www.genelink.com Fax: 914.769.1193

Brief Protocol

A. Genemer™ Reconstitution

Stock Primer Mix: Dissolve the supplied lyophilized Genemer in 100 µl sterile TE. The 10 nmols of primers when dissolved in 100 µl will give a solution of 100 µM i.e. 100 pmols/µl.

Primer Mix: Prepare a 10 pmols/µl Primer Mix solution by a ten fold dilution of the stock primer mix. Example: Add 180 µl sterile TE to a new tube, to this tube add 20 µl of primer stock solution. Label this tube as Primer Mix 10 pmols/µl.

B. Thermal Cycler Files

Protocol for RhD & RhCcEe DNA Genotyping

The following PCR* profile has been optimized for RhD & RhCcEe specific product amplification using the supplied Genemer™.

Program the following thermal cycler files.

1. Hot Start

Rh Hot Start		
Step	Time & Temperature	Cycles
Initial Denaturation	95 °C for 5 minutes	1
Annealing	50 °C Hold Infinity	Hold
Comments: Add Taq premix while on hold.		

2. Amplification File

RhD & RhCcEe Amplification			
Step	Temperature	Time	Cycles
Denaturation	94 °C	30 sec.	30
Annealing	52 °C	30 sec.	
Elongation	72 °C	30 sec.	
Fill in Extension	72 °C	7 minutes	1
Hold	4 °C	Infinity	Hold

C. PCR

1. PCR Premix Preparation (PP). Label tube "PP"

PCR Premix Preparation (PP)		
Component	1 X 50 µl Rxn.	10 X 50 µl Rxns.
Sterile Water	32 µl	330 µl
10 X PCR Buffer	4.5 µl	45 µl
2.5 mM dNTP	5 µl	40 µl
10 pmol/µl Primer Mix	2.5 µl	25 µl
Template DNA (~500 ng)	1-2 µl	Add DNA to each tube
Total Volume	45 µl	
After adding template start hot start PCR File		

Dispense 44µl of the above PCR premix to individual PCR tubes for each amplification reaction and then add the template DNA. Start "Hot Start" thermal cycler file. While holding at 50 °C add 5 µl of the Taq Enzyme Mix (EM). Start amplification file.

2. Taq Polymerase mix Preparation (EM). Label tube "EM"

Taq Enzyme Mix Preparation (EM)		
Component	1 X 50 µl Rxn.	10 X 50 µl Rxns.
Sterile Water	5 µl	50 µl
10 X PCR Buffer	0.5 µl	5 µl
Taq Polymerase	0.5 µl	5 µl
Add 5 µl to each reaction after holding after hot start		

D. Electrophoresis

Load 10 to 15 µl samples to 1.8% agarose gel. Run at 90 mAmps.

E. Results & Interpretation

See Page 1 of this sheet.

Ordering Information

Genemer™ (Selected List) Primer pair for gene or mutation specific amplification. Special optimized conditions may be required for certain amplifications

	Size	Catalog No.	Price, $
Fragile X (spanning CGG triple repeat region)	10 nmole	40-2004-10	100.00
Huntington Disease (spanning CAG triple repeat region)	10 nmole	40-2025-10	100.00
Myotonic Dystrophy (spanning CTG triple repeat region)	10 nmole	40-2026-10	100.00
Friedreich's Ataxia (spanning GAA triple repeat region)	10 nmole	40-2027-10	100.00
Factor V	10 nmole	40-2035-10	100.00
Factor VIII (Hemophilia)	10 nmole	40-2036-10	100.00
STS (Steroid Sulfatase)	10 nmole	40-2023-10	100.00
HGH (Human Growth Hormone)	10 nmole	40-2024-10	100.00
Sickle Cell	10 nmole	40-2001-10	100.00
RhD (RhD gene exon 10 specific)	10 nmole	40-2002-10	100.00
Rh EeCc (Rh Ee and Cc exon 7 specific)	10 nmole	40-2003-10	100.00
Gaucher (various mutations)	10 nmole	40-2047-XX	100.00
Tay Sachs Disease (various mutations)	10 nmole	40-2028-XX	100.00
Cystic Fibrosis (various mutations)	10 nmole	40-2029-XX	100.00
SRY (sex determining region on Y)	10 nmole	40-2020-10	100.00
X alphoid repeat	10 nmole	40-2021-10	100.00
Y alphoid repeat	10 nmole	40-2022-10	100.00

***Please visit www.genelink.com for other Genemer™ not listed here**

**The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents. *Prices subject to change without notice.*

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