Product Specification

HbA, HbS and HbC Genemer™ Specific Control DNA

Sickle Cell Genemer™ Control DNA

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
❑	Sickle Cell Genemer™ HbA Control DNA	40-2001-01	500 ng
❑	Sickle Cell Genemer™ HbS Control DNA	40-2001-02	500 ng
❑	Sickle Cell Genemer™ HbC Control DNA	40-2001-03	500 ng

Shipped at ambient temperature. Store at -20°C

For research use only

Not for use in diagnostic procedures for clinical purposes

Background

The hemoglobin beta, delta and gamma chain genes are on Chromosome 11 and the alpha chains are coded on Chromosome 16. The beta variants such as Hb S, Hb C, and Hb D all occur from mutations on Chromosome 11. The cause of the disorder sickle cell anemia is due to a single base change of A to T in the β globin chain resulting in the substitution of amino acid glutamine to valine at the sixth position. The resulting mutant globin chain is termed as the Hb S. Hemoglobin S is freely soluble when fully oxygenated, under conditions of low oxygen tension the red cells become grossly abnormal assuming a sickle shape leading to aggregation and hemolysis. Homozygous Hb S is a serious hemoglobinopathy found almost exclusively in the Black population. About 8% of American Blacks are carriers and about 0.2% are affected.

Hemoglobin C (Hb C) is due to a single base change of G to A leading to a substitution of lysine for glutamic acid in the sixth position of the β globin chain. Hb C occurs in higher frequency in individuals with heritage from Western Africa, Italy, Greece, Turkey, and the Middle East. There is shortened red cell survival in Hb C homozygotes and sickling complications in compound heterozygotes for Hb S and Hb C.

DNA analysis for the sickle cell mutation is done by specific amplification of the DNA region spanning the mutation using polymerase chain reaction followed by enzymatic cleavage of the amplified product. Sickle cell mutation abolishes a restriction endonuclease site (*Dde* I). Electrophoretic resolution of the fragment pattern reveals the presence or absence of the mutation. Clear genotyping of normal, carrier and homozygous DNA is achieved.

Protocol For Sickle Cell DNA Genotyping

Material Supplied

A tube containing 500 ng of lyophilized control 801 bp DNA segment of the specified Hb gene segment spanning the Sickle Cell mutation region. The above control DNA is an ideal genotyping template for optimizing and performing control amplification with unknown DNA.

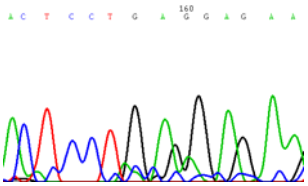
The quantity supplied is sufficient for 1000 regular 50µl PCR** reaction.

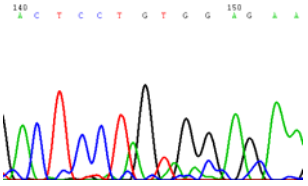
Reconstitution

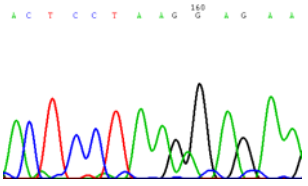
1. **Stock Solution**: Add 100µl sterile water to the tube containing the lyophilized DNA to yield a solution of 5 ng/µl.
2. **Working Solution**: Dilute 1:10 an aliquot of the stock solution.

Usage: Initially use 1µl each of the stock and working template solution for amplification and optimization of the reaction. Based on the results, use 1µl of template at the lowest concentration.

Sequence Information


Hb-A: …TCCTGAGGAG…


Hb-S: …TCCTGTGGAG…


Hb-C: …TCCTAAGGAG…

References:

1. Saiki et al. (1985) Science 230:1350-1354
2. Wu et al. (1989) PNAS 86:2757-2760
3. Conner et al. (1983) PNAS 80:278-282

Recommended Procedure

A. Genemer™ HbS Control DNA [40-2001-02] Reconstitution Protocol

A tube containing 500 ng of lyophilized control 801 bp DNA segment of the specified Hb gene segment spanning the Sickle Cell mutation region. The above control DNA is an ideal genotyping template for optimizing and performing control amplification with unknown DNA.

The quantity supplied is sufficient for 1000 regular 50µl PCR** reaction.

Reconstitution

3. **Stock Solution**: Add 100µl sterile water to the tube containing the lyophilized DNA to yield a solution of 5 ng/µl.
4. **Working Solution**: Dilute 1:10 an aliquot of the stock solution.

Usage: Initially use 1µl each of the stock and working template solution for amplification and optimization of the reaction. Based on the results, use 1µl of template at the lowest concentration.

B. Genemer™ [40-2001-10] Reconstitution Protocol [Not Supplied]

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.

C. Thermal Cycler Files

Amplification Profile

The following amplification profile has been optimized for specific product amplification using the supplied Genemer™.

Program the following thermal cycler files.

1. Hot Start

Hot Start		
Step	Time & Temperature	Cycles
Initial Denaturation	95 °C for 5 minutes	1
Annealing	60 °C Hold Infinity	Hold
Comments: Add Taq premix while on hold.		

2. Amplification File

Amplification File			
Step	Temperature	Time	Cycles
Denaturation	94 °C	30 sec.	30
Annealing	58 °C	30 sec.	
Elongation	72 °C	60 sec.	
Fill in Extension	72 °C	7 minutes	1
Hold	4 °C	Infinity	Hold

D. 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	320 µl
10 X PCR Buffer	4.5 µl	45 µl
2.0 mM dNTP	5 µl	50 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 44 µl 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		

E. Restriction enzyme digestion (100 µl reaction)

Restriction Enzyme Digestion	
Component	Volume
DNA; PCR Reaction	45 µl
10 X Buffer	10 µl
Dde I	10-30 units
Sterile Water	to 100 µl
Digest overnight at 37°C	

Precipitate after overnight digestion; dissolve pellets in 5µl 1 x loading buffer.

F. Agarose Electrophoresis

Load 10 to 15 µl samples to 1.5% agarose gel. Run at 90 mAmps. Confirm correct amplification fragment size.

Results and Interpretation

Mutation abolishes restriction site.

PCR Product Fragment Size 233 bp		
Fragment Sizes After *Dde* I Digestion		
A/A	A/S	S/S
178+55 bp	233+178+55 bp	233 bp

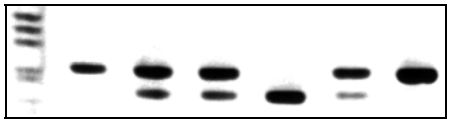


Figure 1. Typical Sickle cell genotype analysis of PCR product digested with *Dde* I. Lane 1 is molecular weight markers. Lane 2 is undigested PCR product. Lanes 3, 4 and 6 is DNA with A/S geneotype. Lane 5 is A/A genotype DNA and Lane 7 represents DNA with S/S genotype.

References:

4. Saiki et al. (1985) Science 230:1350-1354
5. Wu et al. (1989) PNAS 86:2757-2760
6. Conner et al. (1983) PNAS 80:278-282

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

Genemer™ Product Ordering Information

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

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

Genemer™ Control DNA Product Ordering Information

Genemer™ control DNA is a cloned fragment of the mutation region of a particular gene. These control DNA are an ideal genotyping template for optimizing and performing control amplification with unknown DNA.

Product	Size	Catalog No.
Sickle Cell Genemer control DNA (HbA, S and C available)	500 ng	40-2001-0X
GLFX CGG Genemer Control DNA; Fragile X (16, 29, 40, 60 & 90 CGG repeats available)	500 ng	40-2004-0X
GLHD CAG Genemer Control DNA; Huntington Disease (18, 34, 44, 89 & 134 CAG repeats available)	500 ng	40-2025-0X
GLDM CTG Genemer Control DNA; Myotonic Dystrophy (12, 45, 93, 129 & 194 CTG repeats available)	500 ng	40-2026-0X

GeneProber™ Product Ordering Information

The GeneProber™ product line is based on the chemiluminescent Southern blot detection method. Gene Link's non-radioactive detection systems for genotyping of triple repeat disorders are rapid, reliable and as sensitive as the ^{32}P labeled southern blots. No more decayed probes and radioactive exposure. Kits are available for reliable genotyping of the fragile X, myotonic dystrophy and other triple repeat mutation group disorders.

Unlabeled GeneProber™ probes are also available for radio labeling and radioactive based detection. Gene Link strongly recommends the use of non-radioactive gene detection systems. Consider switching to Gene Link's product line of non-radioactive detection systems

Product	Size	Catalog No.
Fragile X GeneProber™ GLFX1 Probe unlabeled	500 ng	40-2004-40
Fragile X GeneProber™ GLFXDig1 Probe Digoxigenin labeled	110 µL	40-2004-41
Huntington's Disease GeneProber™ GLHD14 Probe unlabeled	500 ng	40-2025-40
Huntington's Disease GeneProber™ GLHDDig2X Probe Digoxigenin labeled	110 µL	40-2025-41
Myotonic Dystrophy GeneProber™ GLDM1 Probe unlabeled	500 ng	40-2026-40
Myotonic Dystrophy GeneProber™ GLDMDig2 Probe Digoxigenin labeled	110 µL	40-2026-41
Friedreich's Ataxia GeneProber™ GLFRDA21 Probe unlabeled	500 ng	40-2027-40
Friedreich's Ataxia GeneProber™ GLFRDADig21 Probe Digoxigenin labeled	110 µL	40-2027-41

GScan™ Products Product Ordering Information

Gene Link's GScan™ gene detection products are safe, convenient and sensitive, and afford automated compilation of data. The kits contain optimized PCR amplification reagents and a wide array of fluorescent-labeled primers for genotyping after PCR using fluorescent genetic analyzer instrument(s). Included in these kits are ready-to-run control samples of various repeats of the triple repeat disorder kit. These control samples are for calibration with the molecular weight markers for accurate size determination of the amplified fragments.

The GScan™ kits are simple and robust for routine triple-repeat detection of greater than 100 repeats of all triple repeat disorders listed, except Fragile X. The CGG repeat in Fragile X can be detected up to ~50 repeats.

.

Product	Size	Catalog No.
Fragile X GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2004-15XX
Fragile X GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2004-15FMS
Huntington's Disease GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2025-15XX
Huntington's Disease GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2025-15FMS
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2026-15XX
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2026-15FMS
Friedreich's Ataxia GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2027-15XX
Friedreich's Ataxia GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2027-15FMS

All Gene Link products are for research use only Current pricing are posted at http://www.genelink.com/

Omni-Pure™ DNA & RNA Purification Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Pure™ Blood DNA Purification System	40-4010-01	100
Omni-Pure™ Blood DNA Purification System	40-4010-05	500
Omni-Pure™ Blood DNA Purification System	40-4010-10	1000
Omni-Pure™ Tissue DNA Purification System	40-4050-01	100
Omni-Pure™ Tissue DNA Purification System	40-4050-05	500
Omni-Pure™ Tissue DNA Purification System	40-4050-10	1000
Omni-Pure™ Plant DNA Purification System	40-4060-01	100
Omni-Pure™ Plant DNA Purification System	40-4060-05	500
Omni-Pure™ Plant DNA Purification System	40-4060-10	1000
Omni-Pure™ Viral DNA Purification System	40-3720-01	100
Omni-Pure™ Viral DNA Purification System	40-3720-05	500
Omni-Pure™ Microbial DNA Purification System	40-3700-01	100
Omni-Pure™ Microbial DNA Purification System	40-3700-05	500
Omni-Pure™ Viral RNA Purification System	40-3650-01	100
Omni-Pure™ Viral RNA Purification System	40-3650-05	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Clean™ Gel DNA Purification and Concentration Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Clean™ Gel DNA Beads Purification System	40-4110-10	100
Omni-Clean™ Gel DNA Beads Purification System	40-4110-50	500
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-10	100
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-50	500
Omni-Clean™ DNA Beads Concentration System	40-4130-10	100
Omni-Clean™ DNA Beads Concentration System	40-4130-50	500
Omni-Clean™ DNA Spin Column Concentration System	40-4140-10	100
Omni-Clean™ DNA Spin Column Concentration System	40-4140-50	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Pure™ Plasmid DNA Purification Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Pure™ Plasmid DNA Purification System	40-4020-01	100
Omni-Pure™ Plasmid DNA Purification System	40-4020-05	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

All Gene Link products are for research use only Current pricing are posted at http://www.genelink.com/

Related Products Ordering Information

Taq Polymerase & Master Mix		
Product	**Catalog No.**	**Unit Size**
Taq DNA Polymerase; 400 units; 5 µ/µL; 80 µL	40-5200-40	400 units
Taq PCR Kit; 200 x 50 µL reactions	40-5211-01	200 reactions
Taq PCR Kit with controls; 200 reactions	40-5212-01	200 reactions
PCR Master Mix (2X); 100 x 50 µL reactions (2 tubes x 1.3 mL)	40-5213-01	100 reactions
PCR Master Mix (2X); 200 x 50 µL reactions (4 tubes x 1.3 mL)	40-5213-02	200 reactions

Related Products Ordering Information

PCR Additives & Reagents		
Product	**Catalog No.**	**Unit Size**
Taq DNA Polymerase 300 units; 5 µ/µL; 60 µL	40-5200-30	300 units
PCR Buffer Standard (10 X); 1.6 mL	40-3060-16	1.6 mL
PCR Buffer Mg Free (10 X) ; 1.6 mL	40-3061-16	1.6 mL
Taq Polymerase Dilution Buffer; 1 mL	40-3070-10	1 mL
dNTP 2mM (10X) ; 1.1 mL	40-3021-11	1.1 mL
$MgCl_2$; 25 mM; 1.6 mL	40-3022-16	1.6 mL
Omni-Marker™ Universal Unlabeled; 100 µL	40-3005-01	100 µL
Primer and Template Mix; 500 bp; 40 reactions; 100 µL	40-2026-60PT	100 µL
Nuclease Free Water; 1.6 mL	40-3001-16	1.6 mL
DMSO; 1 mL	40-3031-10	1 mL
TMAC (Tetramethyl ammonium chloride) 100 mM; ; 1 mL	40-3053-10	1 mL
KCl 300 mM; 1 mL	40-3059-10	1 mL
Betaine; 5M; 1 mL	40-3032-10	1 mL

Omni-Marker™		
Product	**Catalog No.**	**Unit Size***
Omni-Marker™ Universal unlabeled; 100 µL	40-3005-01	100 µL
Omni- Marker™ Universal unlabeled; 500 µL	40-3005-05	500 µL
Omni-Marker™ Universal unlabeled; 1 mL	40-3005-10	1 mL
Omni- Marker™ Low unlabeled;100 µL	40-3006-01	100 µL
Omni-Marker™ Low unlabeled; 500 µL	40-3006-05	500 µL
Omni- Marker™ Low unlabeled; 1 mL	40-3006-10	1 mL
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp; 100 µL	40-3062-01	100 µL
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp; 500 µL	40-3062-05	500 µL

All Gene Link products are for research use only Current pricing are posted at http://www.genelink.com/

Southern Blot Buffers& Reagents

Product	Catalog No.	Unit Size
Agarose Tablets, 0.5 gm each 100 Tablets	40-3011-10	100 tablets
Agarose LE Molecular Biology Grade; 100 g	40-3010-10	100 g
Agarose LE Molecular Biology Grade; 500 g	40-3010-50	500 g
Hybwash A, Hybridization Wash Solution; 200 mL	40-5020-20	200 mL
Hybwash B, Hybridization Wash Solution; 100 mL	40-5021-10	100 mL
TAE Buffer; 50X Concentrate; 100 mL	40-3007-01	100 mL
TAE Buffer; 50X Concentrate; 1 L	40-3007-10	1 L
TBE Buffer; 5X Concentrate; 1 L	40-3008-10	1 L
10x Washing buffer; 200 mL	40-5025-20	200 mL
10% Blocking solution; 100 mL	40-5026-10	100 mL
Seq. Loading buffer; 1 mL	40-5027-00	1 mL
10x AP Detection buffer; 100 mL	40-5031-10	100 mL
Lumisol™ I Hybridization Solution; contains formamide; 200 mL	40-5022-20	200 mL
Lumisol™ II Hybridization Solution; for non-toxic hybridizations; 200 mL	40-5023-20	200 mL
Lumisol™ III Hybridization Solution; for oligo probes; 200 mL	40-5024-20	200 mL

Loading Buffers

Product	Catalog No.	Size
Gel Loading Buffer 5X BPB/XC non-denaturing; 1 mL	40-3002-10	1 mL
Gel Loading Buffer 5X BPB/XC non-denaturing; 15 mL	40-3002-15	15 mL
Gel Loading Buffer 10X BPB/XC non-denaturing; 1 mL	40-3003-10	1 mL
Gel Loading Buffer 10X BPB/XC non-denaturing; 15 mL	40-3003-15	15 mL
Gel Loading Buffer 5X Orange G/XC non-denaturing; 1 mL	40-3004-10	1 mL
Gel Loading Buffer 5X Orange G/XC non-denaturing; 15 mL	40-3004-15	15 mL
Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing; 1 mL	40-5027-10	1 mL
Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing; 15 mL	40-5027-15	15 mL
DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer; 1 mL	40-5028-10	1 mL
DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer; 15 mL	40-5028-15	15 mL
RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide; 1 mL	40-5029-10	1 mL
RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide; 15 mL	40-5029-15	15 mL
RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide; 1 mL	40-5030-10	1 mL
RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide; 15 mL	40-5030-15	15 mL

All Gene Link products are for research use only **Current pricing are posted at http://www.genelink.com/**

Notes: