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

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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.

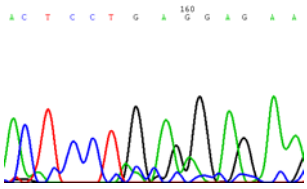
The quantity supplied is sufficient for 1000 regular 50µl PCR** reaction.

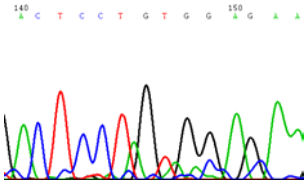
Reconstitution

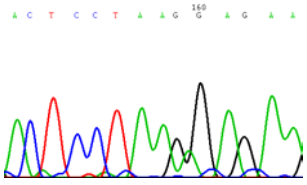
1. **Stock Solution**: Add 100µl sterile water to the tube containing the lyophilized DNA to yield a solution of 5 ng/µl.
2. **Working Solution**: Dilute 1:10 an aliquot of the stock solution.

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







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

**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.

Gene Link™

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Appendix

PCR Premix preparation
Typical Premix

	/50 µl rxn	/1ml
10 x PCR Buffer	4.5 µl	100µl
dNTP mix (2.5mM each) 4 µl	100µl	
Primer Mix (10 pmol/µl each) (25 pmol of each primer/50µl)	2.5 µl	63µl
Sterile water 34 µl	737µl	
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Total	45 µl	1ml

Nucleotide Dilution
Stock: 100 mM; Prepare a final diluted 2.5 mM solution
Preparation

Each 100 mM dNTP	125 µl (Total 500 µl)
Water	4.5 ml

Total volume	5.0ml

Taq Premix ***(per 50µl reaction, scale up as required)***

10 x PCR Buffer		0.5µl
Taq polymerase		0.25µl
Sterile water	4.25µl	

		5µl/rxn.

PCR reaction (50µl)

Diluted DNA(100ng/µl)	1 µl
PCR premix	45 µl
Taq premix	5 µl

PCR products post-processing
1. For oil layered PCR only. Add 200µl of $CHCl_3$ (for both 50µl and 100µl reaction) to each tube, vortex and spin.
2. Transfer the supernatant to fresh eppendorf tube, add 1/10 volume of 3M NaAc (pH 5.2), and 2 volumes of absolute ethanol, precipitate DNA at -80^{o}C for 10 minutes.
3. Spin, rinse the DNA pellet with 700µl of 75% ethanol and dry the pellet in the speedvac.
4. Dissolve the pellet in adequate amount of TE.

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	40-2047-10	100.00
Cystic Fibrosis (various mutations)	10 nmole	40-2029-10	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**

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.

Genemer™ Control DNA (Selected List) Control DNA for use with gene or mutation specific Genemer™

Product	Size	Catalog No.	Price, $
Sickle Cell Genemer control DNA (HbA, S and C available)	500 ng	40-2001-0X	115.00
GLFX CGG Genemer Control DNA; Fragile X (16, 29, 40, 60 & 90 CGG repeats available)	500 ng	40-2004-0X	175.00
GLHD CAG Genemer Control DNA; Huntington Disease (18, 34, 44, 89 & 134 CAG repeats available)	500 ng	40-2025-0X	175.00
GLDM CTG Genemer Control DNA; Myotonic Dystrophy (12, 45, 93, 129 & 194 CTG repeats available)	500 ng	40-2026-0X	175.00

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Prices subject to change without notice.
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