Gene Detection System

# Product Specification

Primer pair for amplification of HbS mutation

# Sickle Cell Genemer™

Catalog No. 40-2001-10 10 nmoles

Shipped at ambient temperature. Store at -20°C

For research use only. **Not for use in diagnostic procedures for clinical purposes.**

## Background

Sickle cell anemia is an autosomal recessive disease. A single base change (A to T) in the β globin chain causes the substitution of amino acid glutamine to valine; the cause of the disorder sickle cell anemia. 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. Heterozygotes (sickle cell trait) are clinically normal, although their red cells will sickle when subjected to very low oxygen pressure in vitro.

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

One tube containing 10 nmoles lyophilized primers [SC2BRC/SC5B]. The quantity supplied is sufficient for 400 regular 50 μl PCR reactions.

### Reconstitution

1. **Stock Primer solution**: Add 50 μl sterile water to the tube containing the primers. The 10 nmoles of primer when dissolved in 50 μl water will give a solution of 200 μM, i.e. 200 pmole/μl.
2. **Primer Mix**: Prepare a 10 pmole/μl Primer Mix solution. Example: Transfer 10μl of stock primer solution to a new tube. Add 190 μl sterile water to this tube. Label this tube as **Primer Mix 10 pmole/μl**.

### A. PCR* reaction (see Appendix for Details)

β globin primers: SC5B and SC2BRC

PCR Profile

| | | |
|---|---|---|
| Denaturation | 94°C | 30 sec. |
| Annealing | 58°C | 30 sec. |
| Elongation | 72°C | 1 min. |

30 cycles, 7 min. 72°C extension, 4°C soak.

### B. Restriction enzyme digestion (100 μl reaction)

| | |
|---|---|
| DNA | 45 μl (45μl of 50 μl PCR reaction) |
| 10 x buffer | 10 μl |
| *Dde* I | 10-30 units |
| Sterile water | up to 100 μl |

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

### C. Electrophoresis

Load samples to 1.5% agarose gel. Run at 90 mAmps for 2.5 hrs.

### D. Results

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 |
| | | |

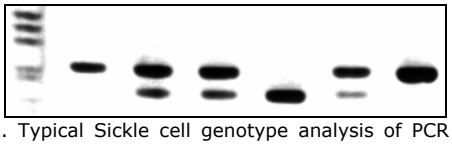

Figure 1. Typical Sickle cell genotype analysis of PCR product digested with *Dde* I. Lane 1 is molecular weight markers. Lane 2 is undigested PCR product. Lanes 3, 4 and 6 is DNA with A/S geneotype. Lane 5 is A/A genotype DNA and Lane 7 represents DNA with S/S genotype.

### References:


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


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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) | 2.5 µl | 63µl |
| (25 pmol of each primer/50µl) | | |
| Sterile water | 34 µl | 737µl |
| | --- | --- |
| 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

| Product | 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 |

***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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