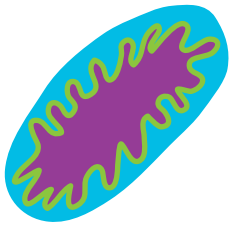
MitoScreen™ Assay Kit

Rapid Mutation Screening of the Entire Mitochondrial Genome Using Multiplex DHPLC

The MitoScreen™ Assay Kit provides the reagents and protocols necessary to screen human genomic DNA populations for heteroplasmic mutations present in the mitochondrial genome. Denaturing high performance liquid chromatography (DHPLC) on the WAVE® System has become a widely recognized technology for both efficient and highly sensitive discovery of known and unknown mutations[1-3].



Human genomic DNA is used as the starting material for amplification of the human mitochondrial genome using a total of 19 primer sets (Figure 1). The amplified overlapping fragments range in size from 300-1500 bp. Four of these fragments are in the size range 300-550 bp and are used for conventional DHPLC analysis. The remaining 15 fragments undergo a restriction digestion step (Figure 2) in order to produce a collection of fragments that are subsequently analyzed by multiplex DHPLC.

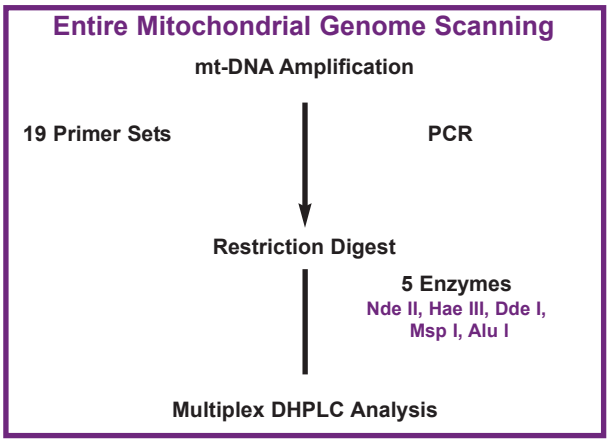


Figure 1: Schematic representation of the MitoScreen Assay Kit Protocol.

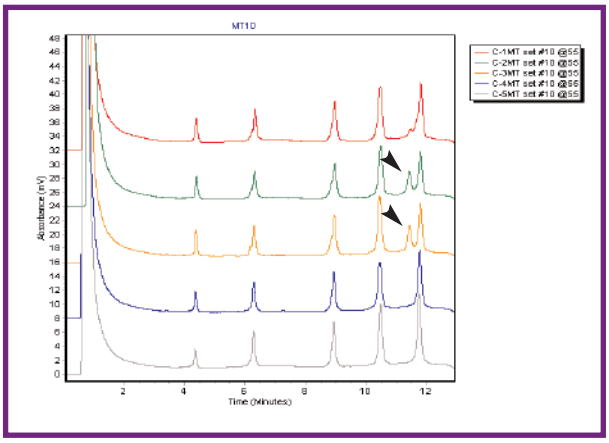


Figure 2: Analysis of PCR fragment MT10 in different pooled DNA samples at 55° C. A heteroplasmic polymorphism is clearly seen in the 5th fragment* of MT10 in DNA samples 2 and 3 as indicated by the arrows.
*Fragment 5 is a 504 bp fragment corresponding to nucleotides 7204-7707 of the mtDNA genome sequence.

Applications

- Mutation screening for mitochondrial disorders, e.g. neuromuscular diseases, cognitive dysfunctions and diabetes
- Mutation screening of mitochondria in relation to cancer research

Contents of the MitoScreen Assay Kit

Each kit contains the following:

- Primer Sets MT 1-19
- Optimase® Polymerase
- PCR reagents and control DNA
- dNTPs
- Restriction enzymes
- MitoScreen Assay Protocol Booklet
- MitoScreen Kit Method CD

Products

Description	Catalog Number	Quantity
MitoScreen Assay Kit	707001	Reagents for 12 DNA samples
MitoScreen Assay Kit Plus	707002	Reagents for 24 DNA samples

Features

Contains all necessary reagents as well as a detailed protocol and a Method CD for screening the entire human mitochondrial genome.

Protocol developed using multiplex DHPLC.

Provides high sensitivity of the WAVE Systems in combination with high fidelity Optimase Polymerase.

All components are WAVE Optimized® and quality controlled.

Benefits

No development work required, all kit components are readily available and compatible with each other and the WAVE System.

Rapid, automated screening of the entire mitochondrial genome in less than 8 hours on a WAVE 3500 and 4500 HT System.

Allows the detection of low levels of heteroplasmic mutations (< 1% heteroplasmy for certain mutations[1]).

Long lifetime for the DNASep® Cartridge, reduced cost and minimized instrument downtime.

Shipping

The MitoScreen Assay Kit is shipped on dry ice. Please store at -15º C to -30º C in a frost-free freezer. Minimum shelf life expected is three months after delivery if stored as directed.

Related Products

Description	Catalog Number
WAVE Optimized HT Pak	SP2012
WAVE Optimized Pak	SP2011
Optimase Polymerase	703030
Premixed dNTP set	705010

Selected References

1. van den Bosch BJC, de Coo RFM, Scholte HR, Nijland JG, van den Bogaard R, de Visser M, de Die-Smulders CEM, Smeets HJM. (2000) Mutation analysis of the entire mitochondrial genome using denaturing high performance liquid chromatography. *Nucleic Acids Research*, Vol. 28, e89.
2. Lui MR, Pan KF, Li ZF, Wang Y, Deng JH, Zhang L, Lu YY. (2002) Rapid screening mitochondrial DNA mutation by using denaturing high-performance liquid chromatography. *World J Gastroenterol*, 8, 426-430.
3. Jones AC, Sampson JR, Cheadle JP. (2001) Low level mosaicism detectable be DHPLC but not by direct sequencing. *Hum Mut* 17, 233-234.

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