SPINAL MUSCULAR ATROPHY SCREENING KIT
Cat. No: 200R-10-03

PRODUCT DESCRIPTION

Spinal muscular atrophy (SMA) is characterized by degeneration of the alpha motor neurons of the spinal cord anterior horn cells, leading to progressive proximal muscle weakness and atrophy. The carrier frequency of SMA from 1/20 to 1/60. The SNP SMA Screening Kit detects the exon 7 deletion with C/T substitution at nucleotide 840 in the SMN1 gene to diagnose the affected states by Quantitative Real Time PCR (qPCR) from blood. The kit analysis homozygous people with % 100 sensitivity and % 100 specificity.

PRINCIPLE OF THE SYSTEM

Test uses 5' Nuclease Assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencer dye only when the probe hybridizes perfectly to the target DNA. This cleavage results in the fluorescent signal which is monitored by Real-Time PCR detection system. An increase in the fluorescent signal (CT) is proportional to the amount of the specific PCR product.

PRODUCT SPECIFICATION

Isolated DNA should be tested with SMA master mix including specific primer and probes for SMN1 Exon 7 and 840 C/T substitution. The system provides all reagents in a **"ready-to-use"** master mix format which has been specifically adapted for qPCR. The dyes of SMA master mix are FAM for SMN1 and TEXAS RED for reference gene.

SYSTEM CONTENTS

Reagents	50 rxns
• SMA Master Mix	1000 µl
• Homozygous Deletion Control DNA*	50 µl
• Wild-Type Control DNA*	50 µl

**** In order to check the compatibility between your Bio-Rad CFX96 Real Time PCR device and SNP SMA Screening Kit, please make a previous study with control DNAs.***

DNA EXTRACTION

The Kit provides high sensitivity with a limit of detection (LOD) level of 0.4 ng/µl. The system is optimized for Macharey-Nagel extraction kit or a similar system. The extracted DNA must be diluted in 150- 200 µl Elution Buffer.

VALIDATION OF DEVICE;

- Leave the master mix and control DNAs at RT to melt.
- Mix the melted master mix gently by pipetting.
- For each samples, pipette **20 µl master mix** into each PCR tubes/strips.
- Add **5 µl Control DNA** into each tube and close the optical caps.
- Run with the programme shown below.
- Control samples should identify expected genotypes for device validation. If they are not, please contact to us for validation of your device (tech@snp.com.tr).
- It is sufficient that the validation of each device should make **only once.**
- After the device validation, the standard test protocol is continued.

STANDARD TEST PROTOCOL;

- Leave the master mix at RT to melt.
- Mix the melted master mix gently by pipetting.
- For each samples, pipette **20 µl master mix** into each PCR tubes/strips.
- Add **5 µl Sample DNA** into each tube and close the optical caps.
- Run with the programme shown below.

PCR PROGRAMME

95 °C	3 Min.	Holding
95 °C	15 Sec.	29 Cycles
60 °C	1 Min.	

Select FAM and TEXAS RED as fluorescent dyes.

This system compatible with;

Bio-Rad CFX96
LightCycler 480 System
ABI 7500 / 7500 Fast
Rotor-Gene Q

DATA ANALYSIS

Before analyzing your results, **set the threshold line to 1500 for both dyes in CFX96.** The threshold line of the other equipments should be adjusted to the fit value.

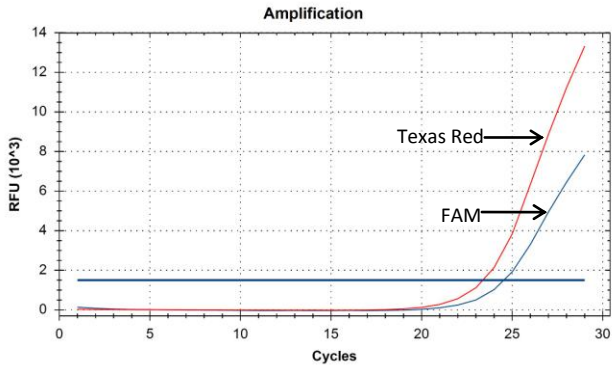

Figure 1: SMA Wild-Type

If the Fam dye and T.Red dye and have amplifications, the sample is **Wild-Type for SMN1 gene.**

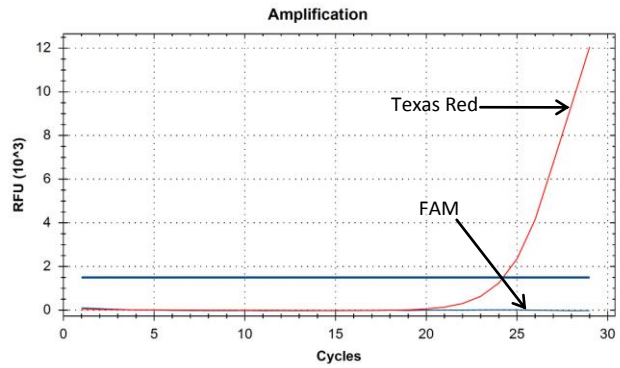

Figure 2: SMA Homozygous Deletion

If the Fam dye has no amplification plot (N/A) and T.Red dye has amplification, the sample is **Homozygous Deletion for SMN1 gene**.

TROUBLE SHOOTING

If there is no amplification in the well,

- Absence of DNA
- Sample is containing DNA inhibitor(s)

Please contact us for your questions. tech@snp.com.tr

CAUTIONS

- Homozygous Deletion samples should be re-tested due to some DNA extraction problems.
- All reagents should be stored at suitable conditions.
- Do not use the PCR master mixes forgotten at room temperature.
- Thaw PCR master mix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR master mix is 12 months. Please check the manufacturing data before use.
- Only use in vitro diagnostics and research.

STORAGE

- All reagents should be stored at – 20 °C and dark.
- All reagents can be used until the expiration date on the box label.
- Repeated thawing and freezing (>5X) should be avoided, as this may reduce the sensitivity of the assay.

REFERENCES

1. American College of Obstetricians and Gynecologists' Committee on Genetics in collaboration with committee members Britton Rink, Stephanie Romero, Joseph R. Biggio Jr, Devereux N. Saller Jr. and Rose Giardine.Committee Opinion. Number 691. March 2017.
2. Shin S1, Park SS, Hwang YS, Lee KW, Chung SG, Lee YJ, Park MH. Deletion of SMN and NAIP genes in Korean patients with spinal muscular atrophy. J Korean Med Sci. 2000;15:93-8.
3. Verhaart IEC, Robertson A, Wilson IJ, Aartsma-Rus A, Cameron S, Jones CC, Cook SF, Lochmüller H. Prevalence, incidence and carrier frequency of 5q-linked spinal muscular atrophy - a literature review. Orphanet J Rare Dis. 2017;12:124.