SPINAL MUSCULAR ATROPHY DETECTION KIT
Cat. No: 200R-10-01

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 and Exon 8 deletion 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 and Exon 8 deletion. 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 Exon 7, HEX for SMN1 Exon 8 and TEXAS RED for reference gene.

SYSTEM CONTENTS

Reagents	50 rxns	20 rxns
• SMA Master Mix	1150 µl	460 µl
• 10X Solution D	500 µl	200 µl
• Homozygous Deletion Control DNA*	30 µl	20 µl
• Wild-Type Control DNA*	30 µl	20 µl

DNA EXTRACTION

The Kit provides high sensitivity with a limit of detection (LOD) level of 0.4 ng/µl.

BLOOD;

The kit is suitable for DNA obtained from spin column and automated extraction systems.

DBS (Dry Blood Spot);

- The system is suitable for DBS samples from newborn (heel blood etc.).
- Prepare fresh **1x Solution D** by diluting the provided **10x Solution D** with PCR Grade water.
- A piece of DBS (punch-3 mm) is put into a 1.5 ml centrifuge tube.
- Add **100 µl of 1x Solution D** onto the punch. Lightly vortexed. Make sure that the punch is completely in the solution.
- Incubate for **5 minutes at room** temperature.
- **2 µl of DNA** is used from the supernatant part.

VALIDATION OF DEVICE;

- Leave the master mix and control DNAs at RT to melt.
- Mix the melted master mix gently by pipetting.
- For each control, pipette **23 µl master mix** into PCR tubes/strips.
- **2 µl** of different **Control DNAs** (homozygous deletion and wild type) and 5-10 **unknown DNAs** belonging to the isolation system to be studied are added to each tube to control the isolation system. Optical caps are closed. Run with the programme shown below.
- Control samples should identify expected genotypes for device validation. If they are not, please contact to manufacturer for validation of your device (tech@snp.com.tr).
- It is sufficient that the validation of each device should be made **only once for each lot.**
- After the device validation, continue with the standard test protocol.

STANDARD TEST PROTOCOL;

- Leave the master mix at RT to melt.
- Mix the melted master mix gently by pipetting.
- For each samples, pipette **23 µl master mix** into each PCR tubes/strips.
- Add **2 µl Sample DNA** into each tube and close the optical caps.
- Run with the programme shown below.

PCR PROGRAMME

96 °C	2 Sec.	32x Cycles for Blood & 40x Cycles for DBS
60 °C	40 Sec	

Select FAM, HEX 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 1000 for all dyes in CFX96.** The threshold line of the other equipments should be adjusted to the fit value.

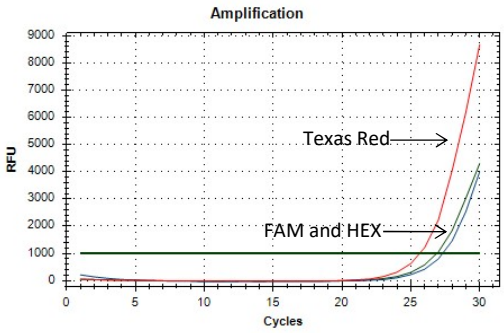

Figure 1: SMN1 Exon 7-8 Wild-Type – Blood Sample

If the FAM, HEX and T.Red dyes and have amplifications, the sample is **Wild-Type for SMN1 gene.**

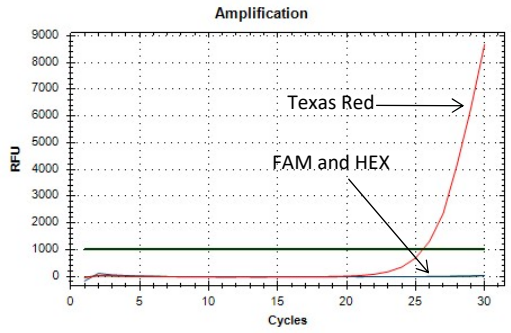

Figure 2: SMN1 Exon 7-8 Homozygous Deletion – Blood Sample

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

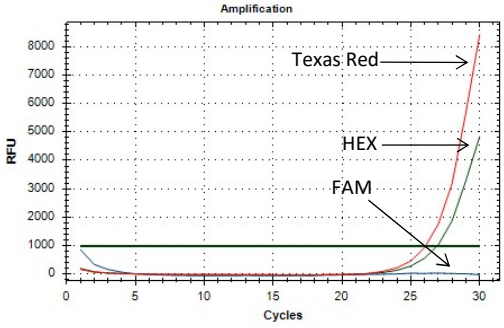

Figure 3: SMN1 Exon 7 Homozygous Deletion – Blood Sample

If the FAM dye has no amplification plot (N/A) and T.Red and HEX dyes has amplification, the sample is **Homozygous Deletion for SMN1 gene for only Exon 7**.

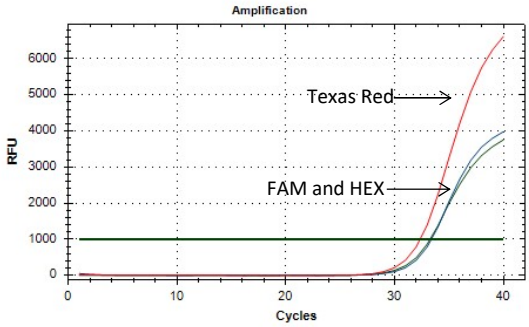

Figure 4: SMN1 Exon 7-8 Wild-Type – DBS Sample

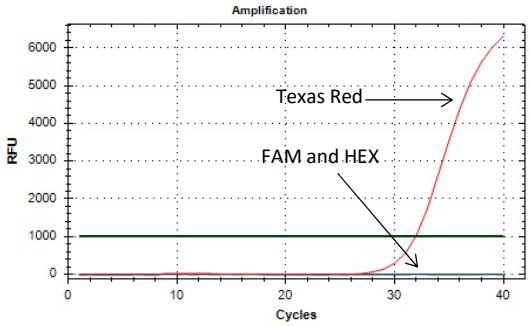

Figure 5: SMN1 Exon 7-8 Homozygous Deletion – DBS Sample

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 24 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. B. Cavdarli,1F. N. Ozturk, S. G. Ergun, M. A. Ergun, O. Dogan and E. F. Percin. "Intelligent Ratio:A New Method for Carrier and Newborn Screening in Spinal Muscular Atrophy". Genetic Testing And Molecular Biomarkers, Volume 24, Number 9, 2020.
2. 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.
3. 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.
4. 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.

CE IV