SPINAL MUSCULAR ATROPHY (SMA) 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. SMA Detection Kit detects the exon 7 deletion with C/T substitution at nucleotide 840 in the SMN1 gene to diagnose the affected states by Real Time PCR (qPCR) from blood and Dry Blood Spot (DBS). 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. Please see table 1 for regions/mutations and dyes.

The system provides all reagents as **"ready-to-use"** which has been specifically adapted for qPCR.

Table 1: Regions/mutations and Dyes

Tube	Regions / Mutations	Dyes
SMA Master Mix	SMN1 Exon 7	FAM
	Reference Gene	HEX

SYSTEM CONTENTS

Reagents	50 rxns	20 rxns
• SMA Master Mix	1000 µl	400 µl
• Solution E	1700 µl x3	1000 µl x2
• Homozygous Deletion Control DNA*	40 µl	20 µl
• Wild-Type Control DNA*	40 µl	20 µl

*Control DNAs are containing plasmids. Amplification plots of control DNAs may appear slightly different from the sample DNAs.

DNA EXTRACTION

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

For Blood Samples;

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

For DBS Samples;

- The system is suitable for DBS samples from newborn (heel blood etc.).
- For DNA isolation from DBS, **Solution E** included in the kit should be used.
- Keep the **Solution E** to melt at room temperature before starting to work. After the first use, the **Solution E** can be stored at Room Temperature.
- Add **1 punch DBS (3 mm)** into 1.5 microcentrifuge tube.
- Mix by inverting the **Solution E** tube and transfer **100 µl** into 1.5 microcentrifuge tube. Make sure that the punch is completely in the solution.
- Incubate at **92 °C for 15 minutes.**
- Use **directly 5 µl** as a Sample DNA.

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

96 °C	5 Sec.	30x Cycles for Blood & 40x Cycles for DBS
60 °C	40 Sec	

Select FAM and HEX as fluorescent dyes.

This system compatible with*;

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

*All the Real-Time PCR equipment with FAM and HEX filter.

DATA ANALYSIS

- The threshold value should be set to **800** for all dyes.
- The ct value for the Reference Gene (HEX) should be **≤ 26** for Blood samples and **≤ 34** for DBS samples**.** Samples that do not comply with this value should be repeated.
- The results can be evaluate by looking at the amplification plots (Figure 1-5).
- The amplification plots below are from the Bio-Rad CFX96 instrument. For other devices, please contact (tech@snp.com.tr).

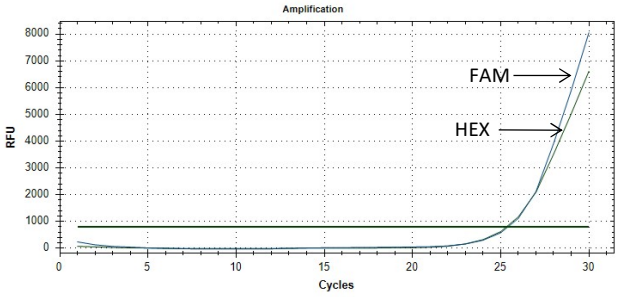

Figure 1: SMN1 Exon 7 Wild-Type – Blood Sample

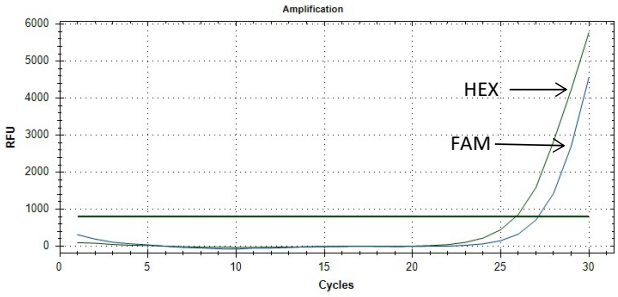

Figure 2: SMN1 Exon 7 Carrier – Blood Sample

If the FAM and HEX dyes have amplification plots, the sample is **Wild-Type or Carrier for SMN1 gene.**

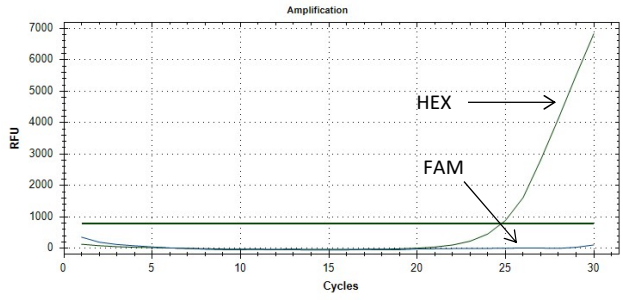

Figure 3: SMN1 Exon 7 Homozygous Deletion – Blood Sample

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

If there is no amplification plots in both FAM and HEX, the sample should be repeated from isolation Step.

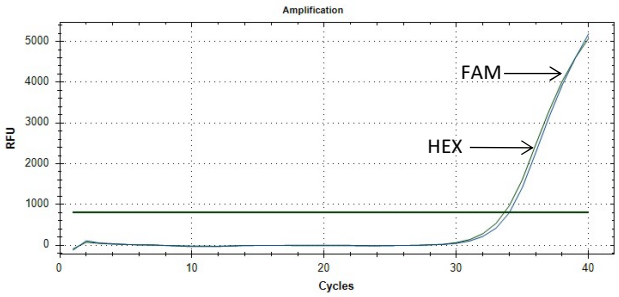

Figure 4: SMN1 Exon 7 Wild-Type – DBS Sample

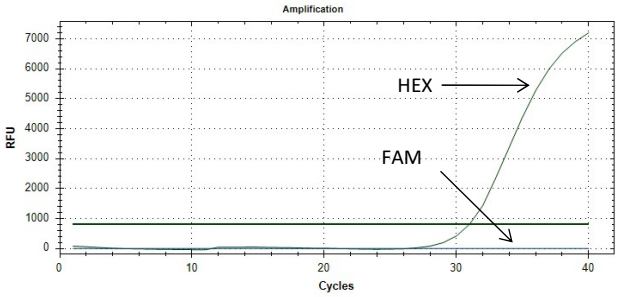

Figure 5: SMN1 Exon 7 Homozygous Deletion – DBS Sample

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.

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