SPINAL MUSCULAR ATROPHY *PLUS* SCREENING 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 *Plus* 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 carrier and affected states by Quantitative Real Time PCR (qPCR) from blood. The kit analysis homozygous and carrier 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. The System is based on **"intelligent ratio (IR)"** between reference and target genes quantifications. **IR** values may vary according to target genes and/or Bio-Rad CFX96 Real Time PCR devices. These varieties should be adjusted in the SMA *Plus* Software settings at validation of device step (see Validation of device and Data Analysis sections).

PRODUCT SPECIFICATION

Isolated DNA should be tested with SMA *Plus* master mix including specific primer and probes for SMN1 Exon 7 and 840 C/T substitution and Exon 8. The system provides all reagents in a **"ready-to-use"** master mix format which has been specifically adapted for qPCR. The dyes of SMA *Plus* master mix are FAM for SMN1 Exon 7, CY5 for SMN1 Exon 8 and TEXAS RED for reference gene.

SYSTEM CONTENTS

Reagents	50 rxns
• SMA *Plus* Master Mix	1000 µl
• Carrier Control DNA*	50 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 *Plus* 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 Kit 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, CY5 and TEXAS RED as fluorescent dyes.

This system is compatible with;

Bio-Rad CFX96

DATA ANALYSIS

- You should use SMA *Plus* Software that can calculate IR values for data analysis. Please check the manual of SMA *Plus* Software.
- The results should be seen like **figure 1.**
- You can also check the results by amplification plots **(Figures 2 to 4).**

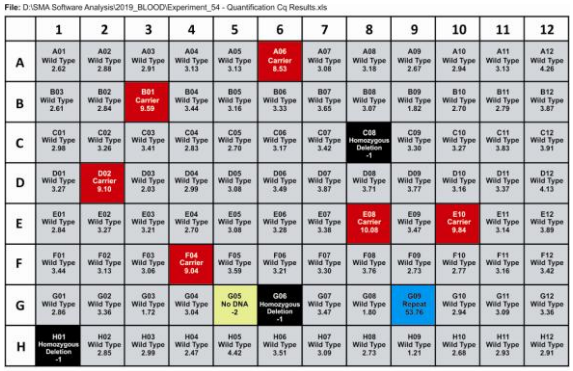

Figure 1: Results by the SMA Plus Software

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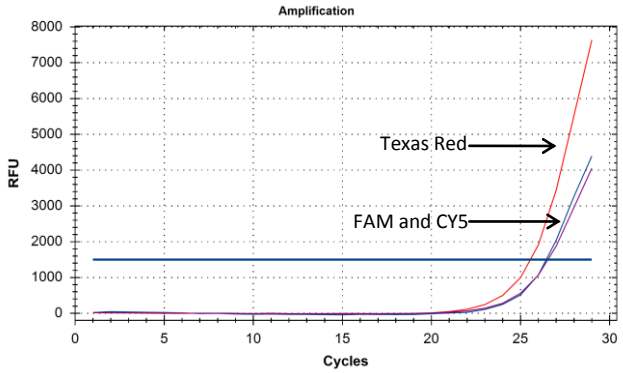

Figure 2: SMN1 Wild-Type Sample

For the SMA carrier state, FAM (SMN1 Exon 7 - blue plot), CY5 (SMN1 Exon 8 - purple plot) and TEXAS RED (Reference gene - red plot) dyes should be analyzed together. If the SMN1 plots are close to the reference gene plot and the values of the **IR** that adjusted to SMA *Plus* Software are less than the limit value, the sample is **Wild-Type for the SMN1 gene.**

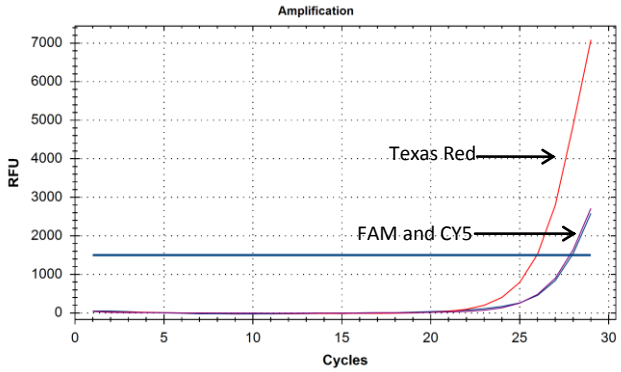

Figure 3: SMN1 Carrier State

If the SMN1 plots are not close to the reference gene plot and the values of the **IR** that adjusted to SMA *Plus* Software are higher than the limit value, the sample is **Carrier state for the SMN1 gene.**

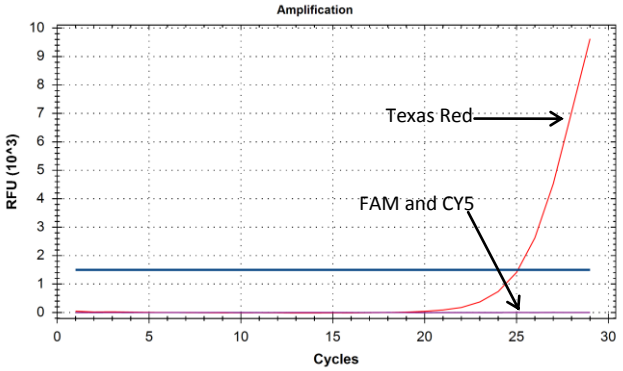

Figure 4: SMN1 Homozygous Deletion

If the Fam dye and CY5 dye have no amplification plots (N/A), 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

Inability in the SMA *Plus* Screening Analysis;

- The SMA *Plus* Screening Kit performs homozygous deletion and carrier screening on Exon 7 and Exon 8 of SMN1 gene. Therefore, the kit does not detect other rare intragenic mutations (2-4%) that cause SMA disease.
- The SMA *Plus* Screening Kit detects the dosage of SMN1 gene. In a normal individual, 2 copies of the SMN1 gene are found (1 + 1). In rare cases (1-4%), there is no SMN1 gene in one chromosome but 2 SMN1 genes can be found in duplicated in the other chromosome (2 + 0). In this case, two copies of SMN1 are reported with the SMN1 Exon 7 screening test. However, the subject is the carrier state for SMA disease.

CAUTIONS

- Homozygous deletion and carrier samples should be re-tested due to some DNA extraction problems. If the sample is SMN1 Exon 8 carrier and Exon 7 Normal, the results should be confirmed by DNA sequence analysis.
- 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.