# Enhanced IntRaFast-Q Sma Screening Kit
## Cat. No: 200R-60-01

### 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. Enhanced IntRaFast-Q SMA Screening Kit detects the Exon 7 and Exon 8 deletion and C/T substitution at nucleotide 840 of Exon 7 in the SMN1 gene to diagnose the carrier and homozygous types by Quantitative Real Time PCR (qPCR) from blood samples. The kit has % 100 sensitivity and % 100 specificity for the detection of above mentioned targets in homozygous and carrier types [(1)].

The 1+1 non-carrier and 2+0 carrier states (Silent Carrier - two SMN1 in the cis) of the SMN1 gene cannot be differentiated by current dosage analysis methods. Therefore, it is difficult to diagnose SMA carrier status in Partners. Recent studies show that the presence of two variants is associated with SMN1 2+0 status depending on the frequency in ethnicity (Figure 1). These variants in the SMN1 gene are c.*3 + 80 T > G corresponding to g.27134 T > G in intron 7 and c.*211_*212del corresponding to g.27706_27707delAT in exon 8. For detecting carriers in SMA Screening with greater sensitivity, Enhanced IntRaFast-Q SMA Screening Kit analyzes these two variations also[(2)].

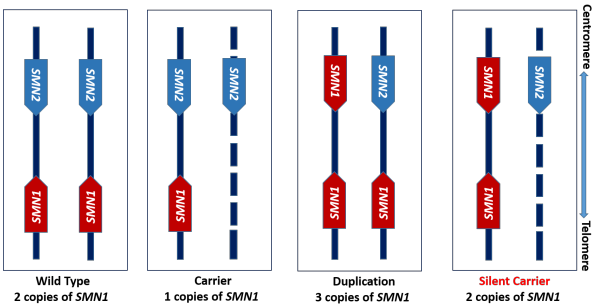


**Figure 1:** Copy number situtuations of SMN1 gene.

### 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 Bio-Rad CFX96 Real Time PCR devices. Therefore IntRa-Q Software should be adjusted to the Bio-Rad CFX96 Real Time PCR device during system setup (see Validation of device and Data Analysis sections).

### PRODUCT SPECIFICATION

Isolated DNA should be tested with IntRaFast-Q master mixes including specific primer and probes for the Exon 7 and Exon 8 deletion and C/T substitution at nucleotide 840 of Exon 7 in the SMN1 gene and c.*3 + 80 T > G and c.*211_*212del variations Please see table 1 for regions/mutations/variations and dyes.

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

**Table 1:** Regions/mutations/variations dyes

| Tube | Regions / Mutations/Variations | Dyes |
|---|---|---|
| **IntRaFast-Q Master Mix 1** | SMN1 Exon 7 | FAM |
| | SMN1 Exon 8 | HEX |
| | Reference Gene | TEXAS RED |
| **IntRaFast-Q Master Mix 2** | 3+80 T>G | HEX |
| | c.*211_*212del | QUASAR 705 |
| | Reference Gene | TEXAS RED |

### SYSTEM CONTENTS

| Reagents | 20 rxn | 50 rxn |
|---|---|---|
| IntRaFast-Q Master Mix 1 | 460 µl | 1150 µl |
| IntRaFast-Q Master Mix 2 | 460 µl | 1150 µl |
| SMN1 Exon 7-8 Wild-Type Control ** | 20 µl | 30 µl |
| SMN1 Exon 7-8 Carrier Control ** | 20 µl | 30 µl |
| SMN1 Exon 7-8 Homozygous Deletion Cont. ** | 20 µl | 30 µl |
| SMN1 3+80 G and 211-212del Positive Control | 20 µl | 30 µl |

* Control DNAs including plasmids were adjusted to extracted DNA. Amplification plots of plasmid control DNA may appear slightly different from the sample DNA.

### DNA EXTRACTION

The Kit provides high sensitivity with a limit of detection (LOD) level of 0.5 ng/µl.

### SPIN COLUMN AND AUTOMATED DNA EXTRACTION SYSTEM;

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

### VALIDATION OF DEVICE;

- Leave the master mixes and control DNAs at RT to melt.
- Mix the melted master mixes gently by pipetting.
- Different tubes should be prepared for each mix.
- For each control, pipette **23 µl master mixes** into PCR tubes/strips.
- **2 µl** of different **Control DNAs** (SMN1 carrier, homozygous deletion, wild-type and SMN1 3+80 G and 211-212del Positive Control) 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 mixes at RT to melt.
- Mix the melted master mixes gently by pipetting.
- Different tubes should be prepared for each mix.
- 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 | 5 Sec. | **35 x Cycles** |
| 60 °C | 40 Sec. | |

Select FAM, HEX, QUASAR 705 and TEXAS RED as fluorescent dyes.

Real Time PCR time is **55 minutes for spin column/ Automated systems**.

- The kit should be only used with the Bio-Rad CFX96 Instrument for detection of SMN1 Exon 7 and Exon 8 Wild-Type / Carrier and Homozygous deletion due to Intra-Q software compatibility.
- For the purpose of only detecting SMN1 Exon 7 and Exon 8 wild-type and Homozygous deletion, when SMN1 carrier analysis is not required, the kit can be used with all devices with FAM, HEX and Texas RED filters.
- For Mix 2, the kit require the device with QUASAR 705.

## DATA ANALYSIS

- The threshold value should be set to **1000** for all dyes.
- The ct value for the Reference Gene (Texas RED) should be **≤ 32**. Samples that do not comply with this value should be repeated.
- For mix 1, you should use IntRa-Q Software that can calculate IR values for data analysis. Please check the manual of IntRa-Q Software.
- The results should be seen like **Figure 2.**
- You can also check the results by amplification plots (Figures 3 to 5).
- For mix 2, amplification plots in HEX and/or QUASAR 705 dyes should be accepted as **"Positive"** for 2+0 variations (Figures 6 and 7).

**File:** D:\SMA Software Analysis\2019_BLOOD\Experiment_54 - Quantification Cq Results.xls

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | A01 Wild Type 2.62 | A02 Wild Type 2.88 | A03 Wild Type 2.91 | A04 Wild Type 3.13 | A05 Wild Type 3.13 | A06 Carrier 8.53 | A07 Wild Type 3.08 | A08 Wild Type 3.18 | A09 Wild Type 2.67 | A10 Wild Type 2.94 | A11 Wild Type 3.13 | A12 Wild Type 4.26 |
| B | B03 Wild Type 2.61 | B02 Wild Type 2.84 | B01 Carrier 9.59 | B04 Wild Type 3.44 | B05 Wild Type 3.16 | B06 Wild Type 3.33 | B07 Wild Type 3.65 | B08 Wild Type 3.07 | B09 Wild Type 1.82 | B10 Wild Type 2.70 | B11 Wild Type 2.79 | B12 Wild Type 3.87 |
| C | C01 Wild Type 2.98 | C02 Wild Type 3.26 | C03 Wild Type 3.41 | C04 Wild Type 2.83 | C05 Wild Type 2.70 | C06 Wild Type 3.17 | C07 Wild Type 3.42 | C08 Homozygous Deletion -1 | C09 Wild Type 3.30 | C10 Wild Type 3.27 | C11 Wild Type 3.83 | C12 Wild Type 3.91 |
| D | D01 Wild Type 3.27 | D02 Carrier 9.10 | D03 Wild Type 2.03 | D04 Wild Type 2.99 | D05 Wild Type 3.08 | D06 Wild Type 3.49 | D07 Wild Type 3.87 | D08 Wild Type 3.71 | D09 Wild Type 3.77 | D10 Wild Type 3.16 | D11 Wild Type 3.37 | D12 Wild Type 4.13 |
| E | E01 Wild Type 2.84 | E02 Wild Type 3.27 | E03 Wild Type 3.21 | E04 Wild Type 2.70 | E05 Wild Type 3.08 | E06 Wild Type 3.28 | E07 Wild Type 3.38 | E08 Carrier 10.08 | E09 Wild Type 3.47 | E10 Carrier 9.84 | E11 Wild Type 3.14 | E12 Wild Type 3.89 |
| F | F01 Wild Type 3.44 | F02 Wild Type 3.13 | F03 Wild Type 3.06 | F04 Carrier 9.04 | F05 Wild Type 3.59 | F06 Wild Type 3.21 | F07 Wild Type 3.30 | F08 Wild Type 3.76 | F09 Wild Type 2.73 | F10 Wild Type 2.77 | F11 Wild Type 3.16 | F12 Wild Type 3.42 |
| G | G01 Wild Type 2.86 | G02 Wild Type 3.36 | G03 Wild Type 1.72 | G04 Wild Type 3.04 | G05 No DNA -2 | G06 Homozygous Deletion -1 | G07 Wild Type 3.47 | G08 Wild Type 1.80 | G09 Repeat 53.76 | G10 Wild Type 2.94 | G11 Wild Type 3.09 | G12 Wild Type 3.36 |
| H | H01 Homozygous Deletion -1 | H02 Wild Type 2.85 | H03 Wild Type 2.99 | H04 Wild Type 2.47 | H05 Wild Type 4.42 | H06 Wild Type 3.51 | H07 Wild Type 3.09 | H08 Wild Type 2.73 | H09 Wild Type 1.21 | H10 Wild Type 2.68 | H11 Wild Type 2.93 | H12 Wild Type 2.91 |

**Figure 2**: Results by IntRa-Q Software

IntRa-Q Software is based on the IR method based on "reference gene quantitation/ SMN1 gene Exon 7 and Exon 8 quantitation" ratio calculation. The quantitation values of the reference and SMN1 gene are calculated with the slope curve embedded in the software, and thus the IR value is determined by the ratio of these values to each other. The software shows the SMA test as carrier, homozygous deletion and wild-type depending on these numerical IR values[1].

To check the amplification plots of SMN1 Exon 7 and Exon 8 wild type, carrier states and homozygous deletion, FAM (SMN1 Exon 7 - blue plot), HEX (SMN1 Exon 8 – green plot) and TEXAS RED (Reference gene - red plot) dyes should be analyzed together.

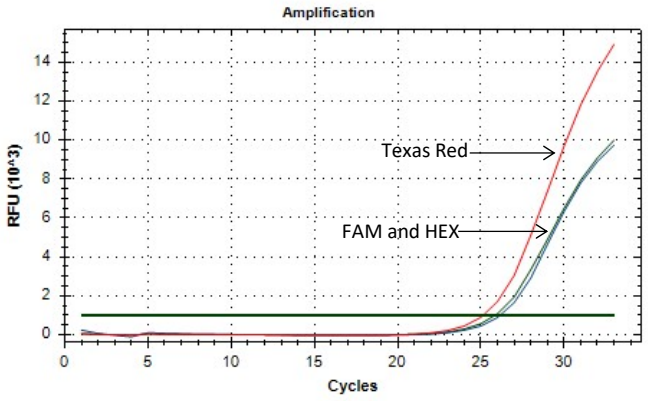


**Figure 3**: SMN1 Exon 7 and Exon 8 Wild-Type Sample (Mix 1)

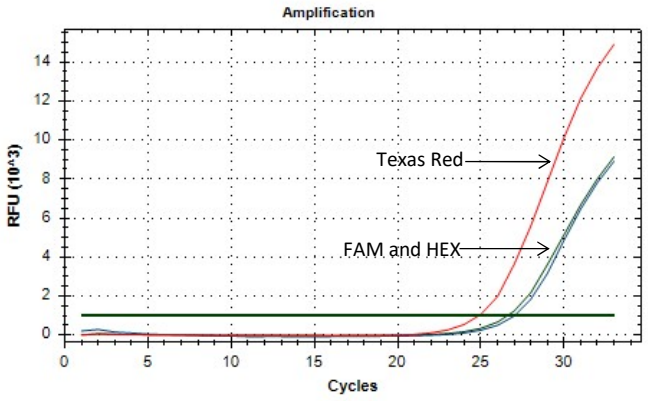


**Figure 4**: SMN1 Exon 7 and Exon 8 Carrier Sample (Mix 1)

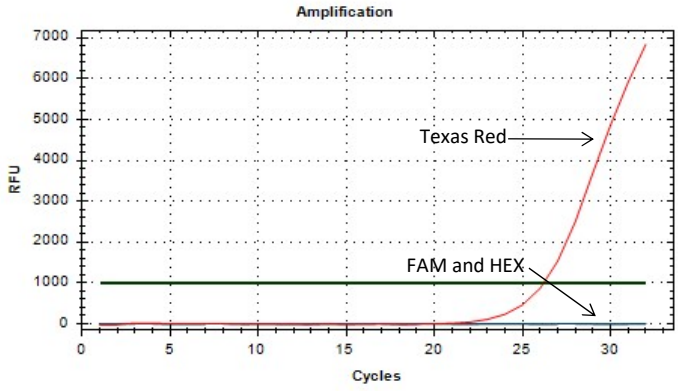


**Figure 5:** SMN1 Exon 7 and Exon 8 Homozygous Deletion (Mix 1)

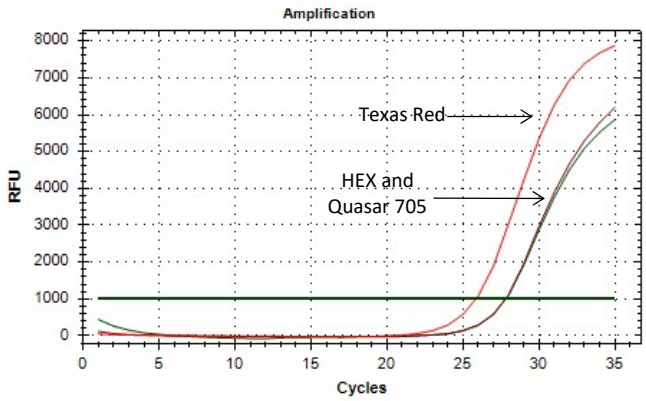


**Figure 6:** SMN1 3+80 T>G and *211_*212del Positive sample (Mix 2)

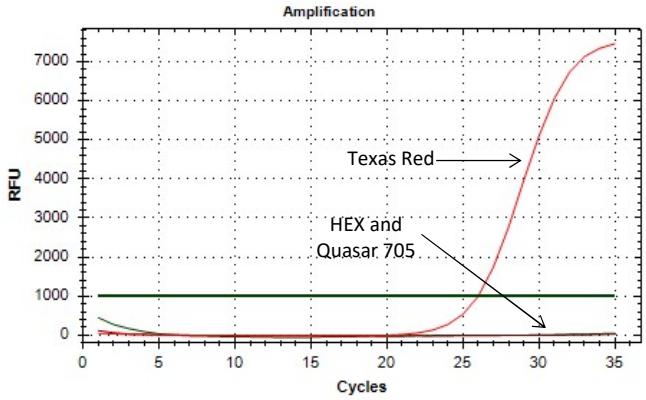


**Figure 7:** SMN1 3+80 T>G and *211_*212del Negative sample (Mix 2)

**Important points to take into account;**

- The results of homozygous deletion and carrier state should be checked from the data analysis screen as amplification plots.
- Some PCR failure problems; like air bubble formation, pipetting etc. may cause unaccepted amplification plots as seen in Figure 8, 9, 10 and 11 it may not be evaluated properly by the software. Please check the amplification plots and re-test the sample if required.
- If the sample is SMN1 Exon 8 carrier and Exon 7 wild-type states, it should be re-tested. In case the results are same, it should be confirmed by DNA sequence analysis.
- If the sample is SMN1 Exon 7 carrier and Exon 8 wild-type states, it should be re-tested for confirmation.

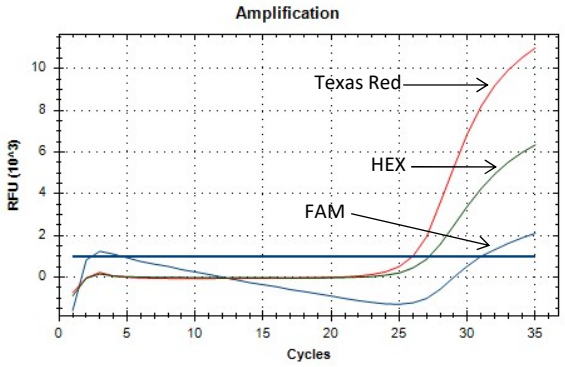


**Figure 8:** Unaccepted amplification plots

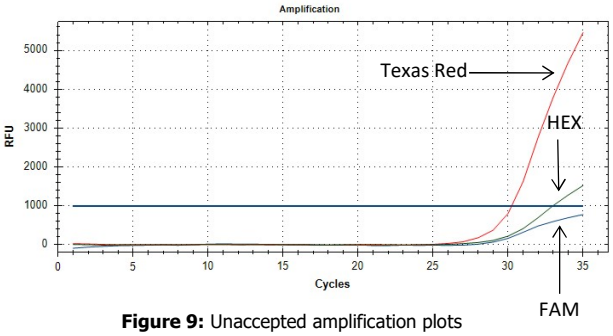


**Figure 9:** Unaccepted amplification plots

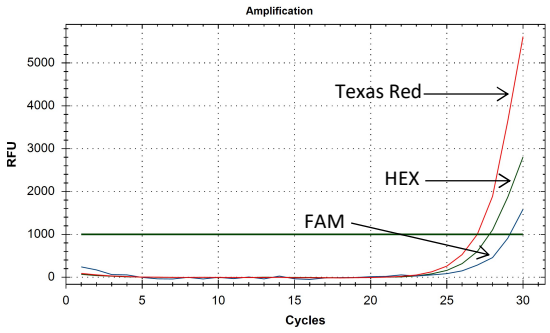


**Figure 10:** Unaccepted amplification plots

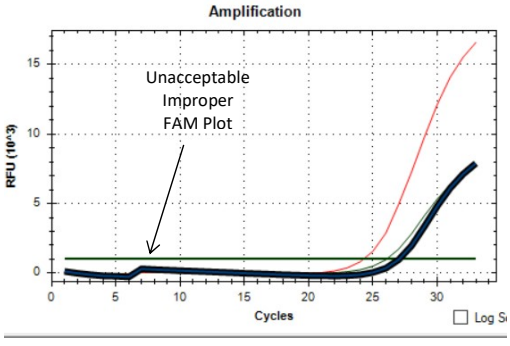


**Figure 11:** Unaccepted amplification plots

**Inability in the Enhanced IntRaFast-Q SMA Screening Analysis;**

- The Enhanced IntRaFast-Q SMA Screening Kit performs carrier state and homozygous deletion 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.

## 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

- All reagents should be stored at suitable conditions.
- Do not use the PCR master mixes forgotten at room temperature.
- Shelf-life of PCR master mix is 24 months. Please check the manufacturing date 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, mentioned 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. Alías L., Bernal S., Calucho M., Martínez E., March F., Gallano P., Fuentes-Prior P., Abuli A., Serra-Juhe C., Tizzano EF. "Utility of two SMN1 variants to improve spinal muscular atrophy carrier diagnosis and genetic counselling". European Journal of Human Genetics (2018) 26:1554–1557
3. 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.
4. 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.
5. 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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