SMN1 RealFast™ Assay

REF 7-700 | 100 reactions

-30°C | -15°C | For research use only.

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1. Intended Use

The SMN1 RealFast™ Assay is a fast and accurate real-time PCR test for the detection of the human *survival of motor neuron 1, telomeric* (*SMN1*) gene variant c.840C>T. Homozygous deletions of exon 7/exon 8 in the *SMN1* gene are highly associated with spinal muscular atrophy (SMA). The qualitative assay discriminates the presence or absence of *SMN1* c.840C in a human genomic DNA extract from blood. This test is not intended for SMA carrier screening. Reference sequence: HGVS: NG_008691.1.

2. Introduction

Spinal muscular atrophy (SMA) is a neurodegenerative disease that affects alpha-motor neurons in the spine. The prevalence of SMA is approximately 1/10.000 with a carrier frequency of 1/40 to 1/60 but may differ significantly in some ethnic groups (1/35 to 1/117). For most SMA cases, the responsible region for the disease is located on chromosome 5q11.2-13.3. The corresponding genes comprise the telomeric survival motor neuron gene 1 (*SMN1*) and the centromeric survival motor neuron gene 2 (*SMN2*). Both genes differ only by a few nucleotides. The *SMN2* gene codes for the same protein as the *SMN1* gene, but SMN2 transcripts are rather unstable because of the variant c.840C>T that causes exon 7 skipping in 90% of SMN2 splicing products. The presence of intact *SMN2* gene copies is a modulator of SMA. However, exon 7 and 8 deletions in the *SMN1* gene are causative for >95% of SMA cases. The absence of exon 7 (Δ7*SMN1*) in both *SMN1* gene copies has a clinical sensitivity of 95% and a clinical specificity of >99% in the diagnostics of SMA.

3. Kit Contents

RealFast™ 4x LIT-Mix	1 vial	white cap	550 µl
SMN1 Assay Mix	1 vial	purple cap	275 µl
SMN1 WT-Control	1 vial	green cap	75 µl
SMN1 MUT-Control	1 vial	red cap	75 µl

The kit contains reagents for 100 reactions in a final volume of 20 µl each.

The RealFast™ 4x LIT-Mix comprises HotStart Taq DNA polymerase and dNTPs in an optimized buffer system. The SMN1 Assay Mix consists of gene-specific primers and dual-labeled hydrolysis probes for *SMN1* and a control gene. Controls representing wild type (WT-Control) and homozygous mutant (MUT-Control) genotypes are supplied with the kit.

4. Storage and Stability

SMN1 RealFast™ Assay is shipped on cooling blocks. On arrival, store the kit at -30 to -15°C. Alternatively, store at 2 to 8°C for short-term use within one month. The kit withstands up to 20 freeze/thaw cycles with no loss of activity. Avoid prolonged exposure to intense light. If stored correctly, the kit will retain full activity until the expiration date indicated on the label.

5. Product Description

5.1. Principle of the Test

The test is based on the fluorogenic 5' nuclease assay, also known as TaqMan® assay. Each reaction contains gene-specific primer pairs that amplify a 142 bp fragment of the *SMN1* gene and a 147 bp fragment of a control gene, the latter serving as the PCR control. Further components are two dual-labeled, gene-specific hydrolysis probes that hybridize to the target sequence of the corresponding fragment. The proximity of the 5'-fluorescent reporter and 3'-quencher dye on intact probes prevents the reporter from fluorescing. During the extension phase of PCR the 5' – 3' exonuclease activity of the Taq DNA polymerase cleaves the 5'-fluorescent reporter from the hybridized probe. The physical separation of the fluorophore from the quencher dye generates a fluorescent signal in real time, which is proportional to the accumulated PCR product.
In samples positive for SMN1 c.840C both, the **FAM-labeled SMN1** probe as well as the **ROX-labeled PCR control** probe bind to the respective gene fragments. A strong fluorescence signal is detected in the FAM channel (520 nm) and the ROX channel (605 nm). In samples negative for SMN1 c.840C, only the ROX-labeled PCR control probe hybridizes to the complementary strand of the control gene fragment. A strong fluorescence signal is detected in the ROX channel and no or only a baseline signal in the FAM channel.

5.2. Real-time PCR Instrument Compatibility

The SMN1 RealFast™ Assay is validated for use with the Bio-Rad CFX 96, Bio-Rad CFX Opus 96, and Applied Biosystems Quantstudio 5 real-time PCR instrumets. The kit is compatible with various common real-time PCR instruments capable of recording FAM and ROX fluorescence:

- ✓ AB QuantStudio 5 (Applied Biosystems)
- ✓ CFX96™ (Bio-Rad)
- ✓ CFX Opus 96

The kit is supplied **without** PCR-grade water.

5.3. Assay Performance Specifications

This prototype of the SMN1 RealFast™ Assay is for research use only.
Analytical performance data: currently not available.
Limit of detection: data not available.
Recommended DNA concentration: 0.2 to 10 ng/µl genomic DNA.

6. Materials Required but not Supplied

Real-time PCR instrument with FAM (520 nm) and ROX (605 nm) filters *), instrument-compatible reaction vessels, disposable powder-free gloves, vortexer, mini-centrifuge for 2.0 ml tubes, tube racks, set of calibrated micropipettes (0.5 - 1000 µl), sterile tips with aerosol-barrier filter, molecular grade water, DNA extraction system, freezer, biohazard waste container.

) The combination of SMN1 RealFast™ Assay with HbS-HbC mpx RealFast™ Assay (REF 7-280) or SCID-XLA mpx RealFast™ Assay (REF 7-710) requires Cy5.5 (711 nm), Cy5 (670 nm), HEX (556 nm), FAM (520 nm) and ROX (605 nm) filters.

7. Experimental Protocol

7.1. DNA Extraction

DNA extraction reagents are **not supplied** with the kit.
Analytical performance data concerning DNA isolated from various specimens (e.g., whole peripheral blood, dried blood spots) is under evaluation and not available yet. Ensure extracted DNA is suitable for amplification in terms of concentration, purity, and integrity.
For accurate genotype calling, the DNA amount per reaction should be within the range of 1 to 50 ng for all samples.

7.2. PCR Controls

Always include the **SMN1 WT-Control** as a positive reference for SMN1 (FAM) and internal control (ROX).
Always include the **SMN1 MUT-Control** as a negative reference and for threshold setting in the FAM channel.

Always include a **No Template Control** (NTC) in each experiment to confirm the absence of potential contamination. It is advisable to run the NTC (use PCR-grade water instead of DNA) in duplicates.

» ***Note:*** *The Controls are potential sources of contamination. Make sure to handle them carefully.* «

7.3. Preparation of SMN1 RealFast™ **Master Mix**

Gently vortex and briefly centrifuge all solutions after thawing. Set up PCR at room temperature. Prepare sufficient **Master Mix** for all your reactions (N samples + positive control + negative controls) plus at least one additional reaction to compensate for pipetting inaccuracies:

Component	per reaction	e.g. 24+1 reactions
RealFast™ 4x LIT-Mix	5 µl	125 µl
SMN1 Assay Mix	2.5 µl	62.5 µl
PCR grade water *)	7.5 µl	187.5 µl
Master Mix	**15 µl**	**375 µl**

*) In case of combination of the SMN1 RealFast™ Assay with the HbS-HbC mpx RealFast™ Assay (REF 7-280) or SCID-XLA mpx RealFast™ Assay (REF 7-710) mix 5µl PCR grade water + 2.5µl SMN1 ASM + 2.5µl HbS-HbC mpx ASM + 5µl 4x LIT Mix, or 5µl PCR grade water + 2.5µl SMN1 ASM + 2.5µl SCID-XLA ASM + 5µl 4x LIT Mix.

Dispense **15 µl Master Mix** into each well. Add **5 µl** purified **DNA** or **Control** template to reach a final reaction volume of 20 µl.
To minimize the risk of contamination, always pipette templates in the following order: first NTC, then samples, and last controls. Immediately close reaction vessels.

» ***Note:*** *Avoid creating bubbles in the final reaction mix and avoid touching the optical surface of the cap or sealing film without gloves. Both may interfere with fluorescence measurements. Centrifuge briefly if needed.* «

7.4. PCR Program

Program the real-time PCR instrument according to the manufacturer's instructions for quantitation experiments with two targets / reporter dyes. Place the samples into the thermal cycler and run the following program:

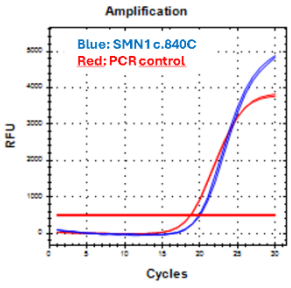
QuantStudio 5, CFX96™, CFX Opus 96, and other Peltier heating block-based instruments

Cycles	Temp	Time	Steps
1	95°C	3 min	Initial denaturation
40	95°C	15 sec	Denaturation
	60°C	1 min	Annealing/Extension – **Data acquisition** in FAM and ROX channel

8. Data Analysis / Interpretation of Results

The presence or absence of the SMN1 c.840C allele is defined by whether there is a signal in the **FAM channel** or not. Successful PCR can be verified by the successful amplification of the control gene detected in the **ROX channel** (PCR control). Genomic DNA samples positive for SMN1 c.840C as well as the **SMN1 WT-Control** show amplification in both the ROX and the FAM channel. SMN1 c.840C negative samples and the **SMN1 MUT-Control** show amplification in the ROX channel only. Fluorescent levels and amplification curves are automatically displayed in amplification plots in the real-time PCR software.

Sample Type	Amplification in **FAM** channel (520 nm)	Amplification in **ROX** channel (605 nm)
SMN1 c.840C positive	**YES**	**YES**
SMN1 c.840C negative	NO	**YES**
SMN1 WT-Control	**YES**	**YES**
SMN1 MUT-Control	NO	**YES**
NTC	NO	NO



Amplification plot:
SMN1 c.840C positive sample.

Some instrument software may need manual threshold settings for accurate analysis.

Recommendations for Threshold Settings (C_q):
Set threshold value for the FAM channel just above the background fluorescent signal generated by the SMN1 WT-Control.
Samples crossing the threshold line beyond C_q 37 give invalid results and must be repeated.

To analyze acquired data, please follow your instrument software instructions.

9. Warnings and Precautions

- For research use only.
- Always use disposable powder-free gloves and wear suitable lab coat when handling specimens and reagents.
- Perform reaction setup in an area separate from nucleic acid preparation and PCR product analysis.
- Use pipettes dedicated for PCR setup only, use aerosol-guarded pipette tips.
- Use instrument-compatible reaction vessels with optically clear caps or sealers.
- Do not mix reagents from different lots.
- Do not use expired kits or kit components.