OCTOBIO

OCTO Health RT-PCR SMN1 Screening Kit

Catalog #	Description
RT-SMN1-25	Real-Time PCR SMN1 Screening Kit, 25 reactions
RT-SMN1-50	Real-Time PCR SMN1 Screening Kit, 50 reactions

IVD

CE

Introduction

Spinal Muscular Atrophy (SMA) is a genetic disease characterized by cell death in the spinal cord's anterior horn cells and brainstem motor nuclei. This disease results in the atrophy of voluntary muscles and often leads to a decline in pulmonary function. A deletion seen in 95% of patients causes this autosomal recessive disorder. The incidence of the disease is 1/6000-1/10000, and the carrier rate in the general population is around 1/50. With these rates, the disease is the second most common pediatric neuromuscular disorder after Duchenne muscular dystrophy.

Diagnosis

Genetic diagnosis of SMA disease is made by detecting the copy number of the SMN1 gene. Individuals with zero copies of the SMN1 gene have SMA disease. Individuals with one copy of the SMN1 gene are considered carriers. Individuals with SMN1 copy numbers other than zero or one are considered healthy. In the "OCTO HEALTH REAL TIME PCR SMN1 SCREENING KIT", diagnosis is made based on the ratio of the peak sizes of the reference gene and SMN1 gene.

The OCTO HEALTH REAL TIME PCR SMN1 SCREENING KIT is used to diagnose the copy number of the SMN1 gene and works with a Real-Time PCR device. The kit works with samples such as heel blood, blood, and tissue after DNA isolation. The kit correlates the copy number of the SMN1 gene with the Reference gene region, which is considered as a reference, according to the "Melt Curve" analysis. The kit can provide results in a single tube with multiplexing using the SybrGreen dye. Depending on the brand and model of the Real-Time PCR device, the OCTO HEALTH REAL TIME PCR SMN1 SCREENING KIT can provide results between 35-45 minutes, making it suitable for screening studies.

Technical Specifications

Diagnosis Method	
Targeted Gene Regions and Fluorescent Dyes	Target Gene: SMN1 (SYBRGreen)
	Reference Gene (SYBRGreen)
Test Tube Count	1
Reaction Volume	20 µl
Test Count	100 sample/300 sample

Kit Contents

	CONTENT	25 RXN	50 RXN
1	SMN1 Master Mix	450 µl	900 µl
2	Positive Control*	25 µl	50 µl
3	Negative Control**	25 µl	50 µl

*Positive Control contains synthetic SMN1 sequences with the Exon 7 deletion (0 SMN1 copies), and therefore produces no melt-peak at 70–71 °C, while the reference gene peak at ~74 °C remains unaffected.
**Negative Control comprises wild-type SMN1 and reference gene templates at equal copy numbers; it yields a melt-peak at 70–71 °C for SMN1 and a corresponding reference peak at ~74 °C with comparable amplitude, confirming assay specificity and performance.

Required Equipment and Materials:

- Compatible RT-PCR Devices*

  Biorad CFX96 and Opus, Qiagene Rotorgene, Quantstudio Real-Time PCR Systems, Roche LightCycler 480 & 96, Mic qPCR Cycler, Corbett Realtime PCR, Slan Realtime PCR, Molarray MA6000, Himedia Insta Q96, Q48 and Q48M
- DNA Isolation (Manual or automated system)
- Vortex and Centrifuge
- Pipette Set (0.5-10µl and 10-100µl)
- Sterile Pipette Tips
- Cold Block
- PCR tubes or plates compatible with the respective Real-Time PCR Device
- +4°C Refrigerator
- Sterile Gloves

Publish Date: September 2021
Doc. Rev. 1.0

** Please contact for other compatible devices*

Storage Conditions

- All components of the kit should be stored at -20°C until the expiration date.
- Kit components should undergo freeze-thaw cycles a maximum of 5 times. If more than 5 freeze-thaw cycles are anticipated, it is necessary to divide the kit components into smaller volumes for storage.
- Negative and Positive Control included in the kit should be stored at 2-8 °C after the initial thawing.

Experimental Preparation

Preparation of Materials:

- Materials stored at -20°C should be thawed on ice or cooling rack.
- Once thawed, liquid materials should be mixed well by pipetting.

Preparation of Reactions:

A single reaction should be prepared for each sample. A separate reaction should be prepared for the Negative Control (NTC) and Positive Control (PC)

Content	Amount (µl)
PCR Master Mix	18
DNA Isolate / PC / NTC	2
TOTAL	20

Real – Time PCR Protocol

Cycle	Temperature	Time	Fluorescence Emission
1	95 °C	300 sec.	
5	95 °C	10 sec.	
	65,5 °C	10 sec.	
	72 °C	3 sec.	FAM
16	85 °C	10 sec.	
	65,5 °C	10 sec.	
	72 °C	3 sec.	FAM
1	95 °C	5 sec.	
1	65 °C	60 sec.	
Melt Analysis			
Initial Temperature	65 °C	Ramp Rate	0,3 °C/sn
End Temperature	84,8 °C	Hold	1 sec.

Peak Height Ratios (SMN1/Reference Gene Region)	Result (SMN1 Copy Number)
0	0 COPY
0.3-0.7	1 COPY
≥0.8	≥ 2 COPIES

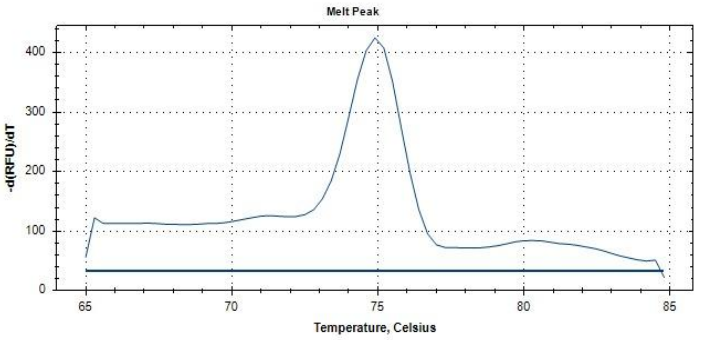
Data Analysis – Interpretation of Results

When interpreting results, refer to the provided tables to:

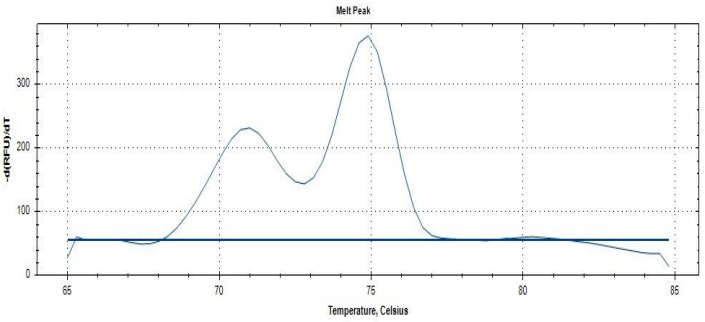
- Determine whether a valid result can be called for the sample under study.
- Classify the sample's SMA status as patient, carrier, or healthy.
- Verify the expected outcomes for the kit's positive and negative controls.

All analyses must be performed using your instrument's "Melt Curve" analysis mode

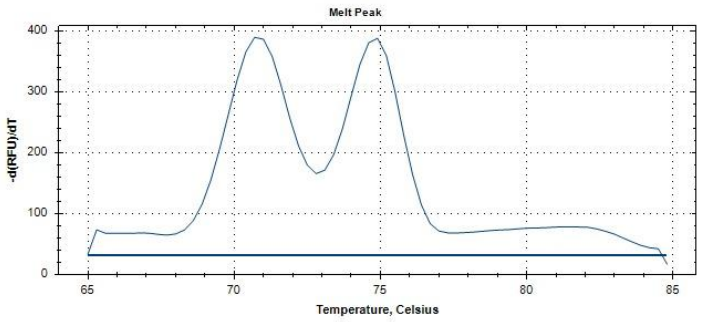
Sample Type	Expected Peak Size Ratios (SMN1/Reference Gene Region)
Positive Sample	0
Negative Sample	>0.8



Sample Image 1: 0 Copy of SMN1 Gene



Sample Image 2: 1 Copy of SMN1 Gene



Sample Image: 2 Copies of SMN1 Gene

- After the Real-Time PCR stage, analysis begins by selecting the Melt Analysis option.
- Each tube must be analyzed separately.
- The analysis should be performed based on the presence or absence of peaks at the specified temperatures for each parameter.

Troubleshooting

Problem: The reference gene melt peak (73–74 °C) is absent. **Possible Cause:** Inefficient or failed DNA isolation. **Suggested Solution:** Repeat the assay starting from the DNA isolation step, ensuring optimal lysis and purification conditions.

Problem: The reference gene melt peak remains absent despite re-isolation.

Possible Cause: Presence of PCR inhibitors in the extracted nucleic acid.

Suggested Solution: Re-isolate DNA using an additional purification or inhibitor-removal protocol. If inhibition persists, assess sample quality (e.g. spectrophotometry, spike-in control) and consider obtaining a fresh specimen.

Problem: A melt peak is observed for SMN1 (70–71 °C) in the positive control (which should lack SMN1). **Possible Cause:** Cross-contamination between work areas, reagents or consumables. **Suggested Solution:** Discard and replace all tubes, tips and plates used. Decontaminate the workspace by wiping with 10 % sodium hypochlorite, allow 30 minutes contact time, then wipe with 70 % ethanol. Prepare new controls in a dedicated clean area.

Problem: Observed peak-height ratios deviate significantly from expected values. **Possible Cause:** Insufficient template quantity or degraded DNA.

Suggested Solution: Repeat from DNA isolation using a higher input volume or more concentrated sample. If deviation recurs, request a new patient specimen.

Tip: Always consult your instrument's maintenance log and ensure that filter sets and heating blocks are within specification before attributing anomalies to the chemistry.

Warnings and Precautions

This test is for in vitro diagnostic purposes only and should be used strictly in accordance with the instructions provided by trained personnel. Clinical samples should be considered as materials potentially carrying an infection risk and should be prepared in a laminar flow cabinet.

• Do not use expired kit. • Avoid repeated freezing and thawing of reagents as it may reduce the sensitivity of the test. • Ensure that reagents are thawed before use, then gently mix the tubes and centrifuge briefly. • Prepare the reaction mixture quickly on ice or a cold block. • Establish two separate work areas, one for isolation and the other for PCR. • Avoid transferring pipettes, tubes, and other working materials between the work areas. • Always use filtered sterile pipette tips. • Use separate lab coats and gloves for each work area.

Symbols Used

The following symbols used on labels and packaging of this product conform to the harmonized standard **TS EN ISO 15223-1**


: **Number of Reactions**


: Expiration Date



: Lot Number



: Reference Number


: Temperature Limit


: Refer to the User Manual


: Manufacturer


: Keep Away From Direct Sunlight

Octo Biotechnology R&D Inc.
REŞİTPAŞA Mah. KATAR Cad. ARI 4 Binası No: 2 /50/6 SARIYER / İSTANBUL ***T:*** 0090 212 286 1368 | +90 555 331 7254
info@octobiotechnology.com www.octobiotechnology.com

Publish Date: September 2021
Doc. Rev. 1.0