# TaqMan SCID/SMA Plus Assay

The Applied Biosystems™ TaqMan® SCID/SMA Plus Assay is a multiplex real-time PCR assay for simultaneous screening of severe combined immunodeficiency (SCID) and spinal muscular atrophy (SMA) directly from dried blood spot (DBS) samples.

Key features

• Optimized assay for SCID and SMA

• Direct extraction from DBS results in less hands-on time

• Compatible with 96- or 384-reaction protocols

• High-quality results for 96- or 384-well format with less than 45 min qPCR run time using fast cycling protocol

• Minimal liquid-dispensing steps for easy automation

Background

Intensive research in early detection of SCID and SMA is essential for prevention of permanent disabilities or death of infants. SCID is a primary immunodeficiency disorder characterized by lack of functional T and B cells, resulting in reduced cellular and humoral immunity. Several genes may be involved, so analysis is based on measurement of episomal DNA products resulting from immune cell development (Figure 1). SMA is an autosomal recessive disorder characterized by degeneration of motor neurons in the spinal cord and brain, leading to progressive muscular weakness. The disease is caused by defects in the *SMN1* gene, which codes for a protein necessary for normal functioning of motor neurons (Figure 2). Using DBS samples as starting material, the TaqMan SCID/SMA Plus Assay is designed to concomitantly detect KREC and TREC (for SCID) and *SMN1* (for SMA), and it includes RNase P as an internal genomic reference.
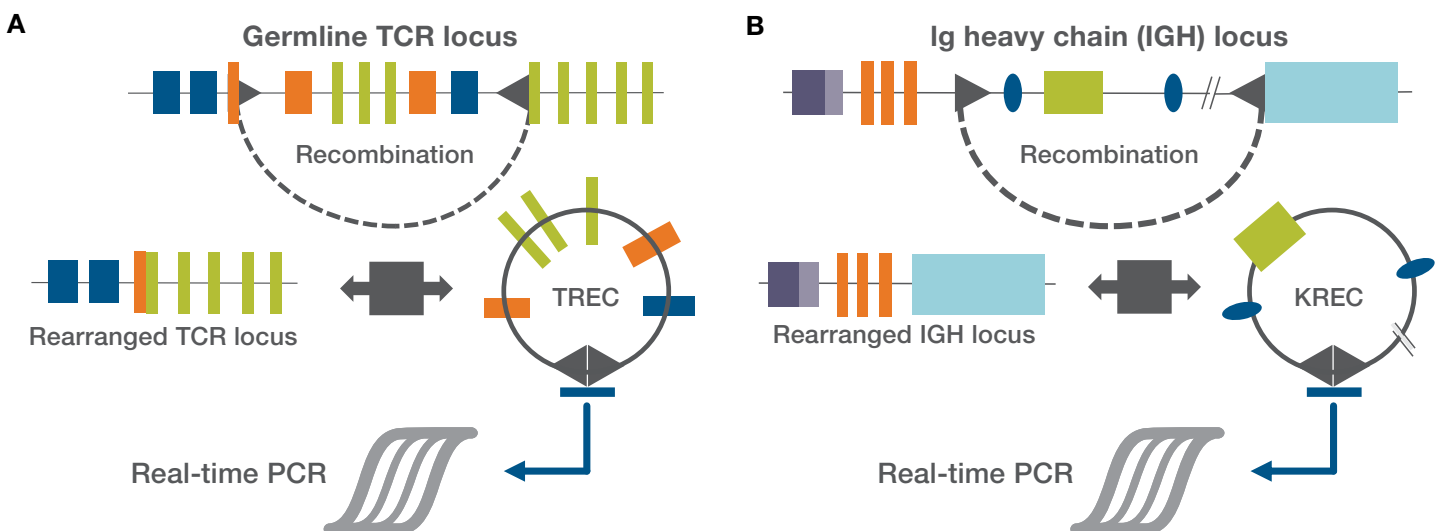
Figure 1. Real-time PCR assay for SCID. (A) T cell receptor excision circles (TREC) are formed during recombination of the T cell receptor (TCR) locus. **(B)** Kappa-deleting recombination excision circles (KREC) are formed during B cell development through recombination of the Ig heavy chain (IGH) locus. Mutation in genes involved in SCID lead to reduced amounts of TREC and KREC, which are measured by real-time PCR.

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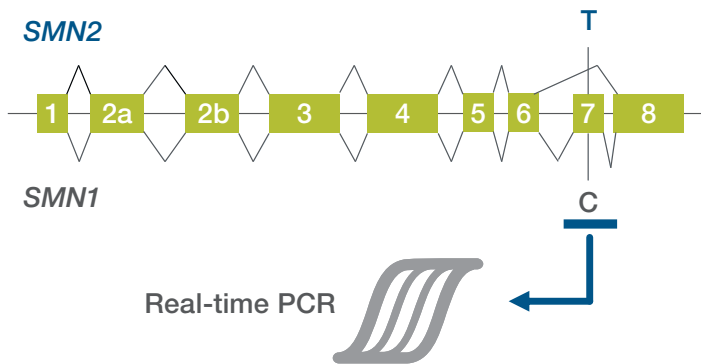
Figure 2. Real-time PCR assay for SMA. In most cases, SMA is caused by homozygous deletion of the *SMN1* gene or by gene conversion to its paralog *SMN2*, which produces a far less functional protein. *SMN1* and *SMN2* differ by five bases, including a single coding base pair (exon 7 C>T). This region in exon 7 is used to detect deletion of *SMN1* or gene conversion to *SMN2* by real-time PCR.

Complete research solution from DBS to result

The TaqMan SCID/SMA Plus Assay detects *SMN1*, TREC, and KREC, as well as the RNase P gene as an internal genomic control. The assay solution contains a mixture of primers and probes for each target plus an *SMN2* blocker. With only 4 liquid-dispensing steps, the DBS sample preparation workflow requires less than 40 min of hands-on time for desiccated DBS samples, or less than 20 min when using freshly prepared, routine DBS samples (Figure 3). Protocols for both 96- and 384-well plates are available, making this a scalable assay adaptable to high-throughput environments. A real-time PCR instrument with at least 5 filters is required to detect the reporter dyes plus the passive reference dye (Table 1).

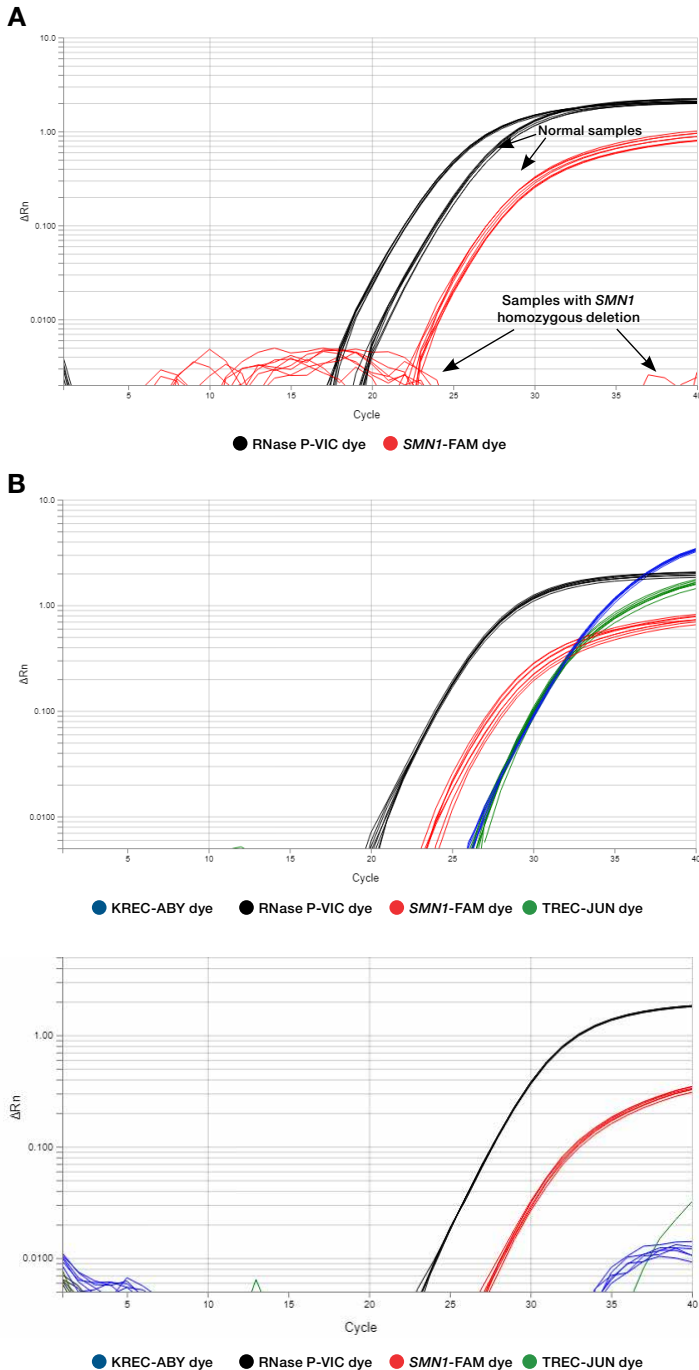| Wash	Extraction	Transfer	Reaction setup	Run
Wash DBS	Direct DNA extraction from DBS	Transfer to a new 96-well or 384-well plate	Add master mix and TaqMan SCID/SMA Plus Assay	Process on QuantStudio instrument with Fast cycling protocol

Figure 3. Workflow for sample preparation and qPCR reaction setup. The DBS punch in each well is either a 1.5 mm or 3.2 mm punch. The Applied Biosystems™ DNA Extract All Reagents Kit contains a lysis solution for processing the DBS samples directly in each well. The DNA extracts from the DBS punches are transferred to new wells on a fresh plate. The Applied Biosystems™ TaqPath™ ProAmp™ Multiplex Master Mix and TaqMan SCID/SMA Plus Assay are combined and added to each well, and plates are run on an Applied Biosystems™ QuantStudio™ Real-Time PCR System.

Table 1. Reporter dyes and recommended instruments.

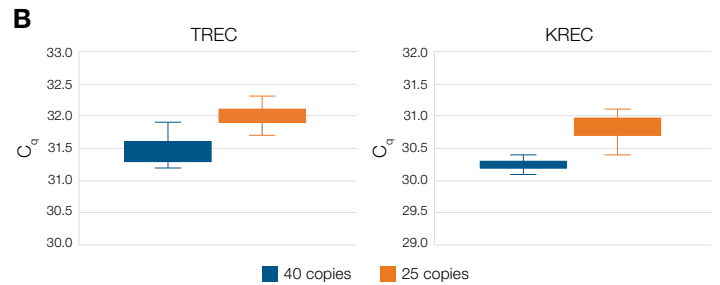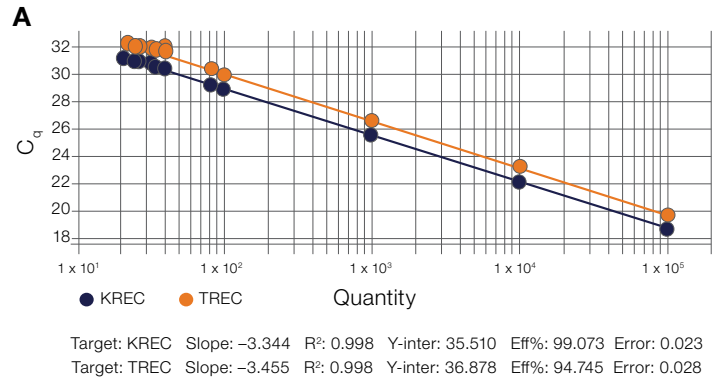| Target	Reporter	Passive reference	Recommended instruments
SMN1	FAM dye		
TREC	JUN dye	Mustang Purple dye	QuantStudio 5, 6 Flex, 7 Flex, Dx (in RUO mode), or 12K Flex Real-Time PCR System
KREC	ABY dye		
RNase P	VIC dye		

Assay results and performance

The TaqMan SCID/SMA Plus Assay is a multiplex real-time PCR assay that detects TREC, KREC, and exon 7 of *SMN1* (Figure 4). The assay includes the RNase P gene as an internal amplification control to verify successful sample extraction.

A

● RNase P-VIC dye   ● *SMN1*-FAM dye

B

● KREC-ABY dye   ● RNase P-VIC dye   ● *SMN1*-FAM dye   ● TREC-JUN dye

● KREC-ABY dye   ● RNase P-VIC dye   ● *SMN1*-FAM dye   ● TREC-JUN dye

Figure 4. Detection of TREC, KREC, and *SMN1* using a multiplex TaqMan SCID/SMA Plus Assay. (A) *SMN1* amplification curves are detected for normal samples. Samples with homozygous deletion of *SMN1* show undetermined *SMN1* C_q values. RNase P amplification curves with the lower C_q correspond to the *SMN1* homozygous deletion sample. **(B)** TREC and KREC amplification curves reproducibly cluster around the same C_q for normal samples (upper graph), while TREC- and KREC-depleted samples result in undetermined TREC and KREC C_q values (lower).

To assess the limit of quantification of the assay for TREC and KREC, 20 replicates of 40 and 25 copies per reaction in a final volume of 20 µL were evaluated. The assay showed accurate quantification of 25 copies per reaction (Figure 5).

Additionally, the assay is optimized for high specificity for *SMN1*, eliminating cross-detection of *SMN2*.

A

● KREC   ● TREC   Quantity

Target: KREC   Slope: –3.344   R²: 0.998   Y-inter: 35.510   Eff%: 99.073   Error: 0.023
Target: TREC   Slope: –3.455   R²: 0.998   Y-inter: 36.878   Eff%: 94.745   Error: 0.028

B

■ 40 copies   ■ 25 copies

	40 copies		**25 copies**	
Target	**TREC**	**KREC**	**TREC**	**KREC**
C_q mean	31.5	30.3	32.0	30.8
C_q SD	0.17	0.14	0.14	0.18

Figure 5. Consistent detection of low concentrations of TREC and KREC. (A) Standard curve for each target. **(B)** Box plots showing distribution of C_q values obtained over 20 replicates for TREC and KREC targets at 40 and 25 copies (per reaction). C_q mean and standard deviation (SD) for each set of replicates are provided in the table.

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Ordering information

Product	Quantity	Cat. No.
TaqMan SCID/SMA Plus Assay	1,000 reactions	A48566
	4,000 reactions	A48567
	8,000 reactions	A48568
	20,000 reactions	A48569
QuantStudio Real-Time PCR System	1 instrument	Various*
On-site training	1 day	A48619

* Assay is compatible with Applied Biosystems™ QuantStudio™ 5, 6 Flex, 7 Flex, 12K Flex, and Dx (in RUO mode) systems.

For additional instrument options, go to **thermofisher.com/quantstudio**

Find out more at **thermofisher.com/NBS**

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