TaqMan™ SCID/SMA Assay

Catalog Numbers A47927, A47928, A47929

Pub. No. MAN0018922 Rev. C.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqMan™ SCID/SMA Assay User Guide* (Pub. No. MAN0018921). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference is intended as a benchtop reference for experienced users of the TaqMan™ SCID/SMA Assay. For detailed instructions and ordering information for additional products, see the *TaqMan™ SCID/SMA Assay User Guide* (Pub. No. MAN0018921).

Contents and storage

Table 1 TaqMan™ SCID/SMA Assay

Cat. No.	Number of reactions	Storage
A47927	4,000	• 2–8°C for up to 3 months • –30°C to –10°C for long-term storage
A47928	8,000	
A47929	20,000	

Procedural guidelines

- Keep the PCR Reaction Mix on a chilled block during real-time PCR reaction setup.
- The PCR Reaction Mix can be formulated up to 48 hours before sample preparation and stored at 2–8°C. See "Prepare the real-time PCR reactions" on page 2.
- Ensure that the instrument is calibrated for each detector dye and passive reference dye, according to the instrument user guide. For more information, see the *TaqMan™ SCID/SMA Assay Calibration Guide* (Pub. No. MAN0019378).

Before you begin

DBS wash buffer and stabilizing buffer are stable at room temperature and can be prepared several days in advance. They do not need to be prepared fresh.

- Prepare DBS wash buffer—add 5 g of liquid Thesit™ reagent into 100 mL of luke warm 1X PBS to prepare a 5% stock, then perform serial dilution using luke warm 1X PBS to prepare 0.5% DBS wash buffer.

  DBS wash buffer is 1X PBS and 0.5% (w/v) Thesit™ reagent.

  Note:
  - Alternatively, a higher or lower concentration and/or volume of stock can be prepared based on the throughput of your laboratory.
  - Heat the Thesit™ reagent until liquid and the 1X PBS until luke warm to allow the Thesit™ reagent to dissolve readily.

- Prepare stabilizing buffer—add 0.5 mL of 10% Tween™-20 Surfact-Amps™ Detergent Solution to 100 mL of TE Buffer.

  Stabilizing buffer is modified TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8) with 0.05% Tween™-20 detergent added.

Extract the DNA

1. Place a DBS punch (1.5 mm or 3.2 mm) from a blood card into the well of the 0.2 mL optical 96-well reaction plate.

   Note: Use only one punch per well.

2. Wash the DBS punch.
   a. Add the DBS wash buffer to each sample well.
      - 1.5 mm DBS punch: 70 µL
      - 3.2 mm DBS punch: 150 µL
   b. Seal the plate with a clear adhesive film, then centrifuge at 2400 rpm for 1 minute to wet and submerge the punch.
   c. Shake the plate for 30 minutes at 1500 rpm on a microplate shaker.
   d. Centrifuge briefly to collect the contents at the bottom of the wells, then remove and discard the supernatant.
   e. Add 150 µL of water to each sample well, then remove and discard the water.
3. Add 5 µL of DNA Extract All Reagents Kit Lysis Solution to each sample well.
4. Seal the plate, then centrifuge briefly to collect the contents at the bottom of the wells.
5. Incubate the reaction plate for 5 minutes at 95°C.
6. Centrifuge briefly to collect any condensed droplets at the bottom of the wells, then cool to room temperature before opening the seal.

   Note: Optionally, place the plate on a chilled block to quickly cool the plate.
7. Add 35 µL of stabilizing buffer to each sample well.

   Note: The total lysate volume per 1.5 mm or 3.2 mm DBS punch is 40 µL.
8. Seal the plate, vortex briefly, then centrifuge briefly to collect the contents at the bottom of the wells.
9. Transfer the lysate to a fresh optical reaction plate according to the following table.

DBS punch	Lysate volume per well	Reaction plate
1.5 mm	9 µL	96-well (0.1 mL or 0.2 mL)
3.2 mm	9 µL	96-well (0.1 mL or 0.2 mL)
3.2 mm	6.75 µL	384-well

Prepare the real-time PCR reactions

1. Prepare the PCR Reaction Mix in an appropriately sized microcentrifuge tube according to the following table.

Component	Volume per reaction[1]	
	96-well plate	384-well plate
2X TaqPath™ ProAmp™ Multiplex Master Mix	10 µL	7.5 µL
20X TaqMan™ SCID/SMA Assay	1 µL	0.75 µL
Total PCR Reaction Mix volume	**11 µL**	**8.25 µL**

[1] Add 10% overage for pipetting loss. Excess PCR Reaction Mix can be stored at 2–8°C for up to 48 hours.

2. Vortex to mix, then centrifuge briefly to collect the contents at the bottom of the tube.
3. Add the PCR Reaction Mix to each well containing the transferred lysate.
   - 96-well plate: 11 µL
   - 384-well plate: 8.25 µL
4. Seal the plate with an optical adhesive film, vortex to mix, then centrifuge briefly to collect the contents at the bottom of the wells.

Set up and run the real-time PCR instrument

See your instrument user guide for detailed instructions to program the thermal cycling conditions or to run the plate.

Note: The instrument must be configured with the block appropriate for the plate type.

1. Select or create dye detectors, then assign them to each well in the plate layout.

Target	Reporter	Quencher
SMN1	FAM™ dye	MGB-NFQ
TREC	JUN™ dye	QSY™
RNase P	VIC™ dye	QSY™

2. Set up the following conditions.
   - Cycling mode: Fast
   - Passive reference: MUSTANG PURPLE™ dye

3. Set the reaction volume.
   - Reaction volume for a 1.5 mm DBS punch in a 96-well plate: 20 µL
   - Reaction volume for a 3.2 mm DBS punch in a 96-well plate: 20 µL
   - Reaction volume for a 3.2 mm DBS punch in a 384-well plate: 15 µL

4. Set up the following thermal cycling protocol.

Step	Temperature	Time	Cycles
Initial denaturation / Enzyme activation	95°C	20 seconds	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

5. Load the plate into the real-time PCR instrument.

6. Start the run.

Analyze the results

See your instrument user guide for more detailed information about data analysis.

1. View the amplification plots.

2. Set the baseline and threshold values.

3. Calculate the C_t values on the instrument.

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Revision history: Pub. No. MAN0018922

Revision	Date	Description
C.0	20 August 2020	Updated wash steps.
B.0	10 August 2020	• Added references to the *TaqMan™ SCID/SMA Assay User Guide* and the *TaqMan™ SCID/SMA Assay Calibration Guide*. • Removed required materials not supplied. • Updated quencher and disclaimer.
A.0	6 April 2020	New document.