Preparation of PCR Master Mix

- PCR Mix: contains Septin 9 primers, blocker and probe; ACTB primers and probe; dNTPS, buffer, $MgCl_2$, water, 24.04 µl/well
    - Oligonucleotide information see Table 1
    - 10 mM dUTP mix
    - 1 M $MgCl_2$
- Polymerase: Ready-to-use Taq Polymerase (5 Units/ µl), 0.96 µl/well
- For a single determination 27.7 µl PCR Mix and 1.1 µl Taq Polymerase are required (extra for pipetting loss).

Supplemental Table 1. Oligonucleotide Sequences and Concentrations

PCR	Forward Primer	Reverse Primer	Blocker	Probe
SEPT 9	AAATAATCCCATC CAACTA	GATT-DS-GTTGTTTATTAGTTAT TATGT	GTTATTATGTTGGA TTTTGTGGTTAATG TGTAG-C3	6FAM-TTAACCGCGAAATCCGA C-BBQ
ASSAY CONC	0.3µM	0.3µM	1.0 µM	0.1 µM
ACTB	GTGATGGAGGAG GTTTAGTAAGTT	CCAATAAAACCTACT CCTCCCTTAA		Rhodamin 6G-ACCACCACCCAACACAC AATAACAAACACA-BBQ
ASSAY CONC	0.9 µM	0.9 µM	N/A	0.1 µM

Supplemental Table 2: Preparation of PCR Master Mix (MM)

Component	Volume for 8 PCRs (4 determinations)	Volume for 12 PCRs (6 determinations)	Volume for 24 PCRs (12 determinations)	Volume for 48 PCRs (24 determinations)
PCR Mix	221.2 µl	331.7 µl	663.5 µl	1327.0 µl
Taq Polymerase	8.8 µl	13.2 µl	26.5 µl	53.0 µl

Supplemental Table 3: Recommended Cycling Program

Program Parameter	Denaturation	Cycling			Cooling
Analysis Mode	None	Quantification mode			None
Cycles	1	50			1
Segment	1	1	2	3	1
Target [°C]	94	93	62	57	40
Hold [hh:mm:ss]	00:30:00	00:00:10	00:00:05	00:00:30	00:00:30

LC 480*	Ramp Rate [°C/s]	4.4	2.2	2.2	2.2	2.2
	Acquisition Mode	None	None	None	Single	None

* LightCycler® 480 System: select the Dual Color Hydrolysis Probe/UPL Probe as detection format, activate the filter combination 483 – 533 nm and 523 – 568 nm. For LightCycler® 480 II activate the filter combination 465 – 510 nm and 533 – 580 nm.