Check the product label for actual catalog number, lot and expiry date.

SampleIN™ UDG 1Step RT qPCR Probe ROX L Mix, 4X

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
DOS0401	200 r of 20 µl	1 ml - SampleIN™ UDG 1Step RT qPCR Probe ROX L Mix, 4X 3 x 1 ml - PCR Water	4X concentrated one-step RT qPCR master mix with Reverse Transcriptase, RNase Inhibitor, Hot Start Taq DNA Polymerase, dNTPs, magnesium and optimized buffer with enhancers and stabilizers. Includes ROX at low concentration. Includes dUTP and UDG (uracil-DNA glycosylase) to prevent PCR carryover contamination.
DOS0405	1000 r of 20 µl	5 x 1 ml - SampleIN™ UDG 1Step RT qPCR Probe ROX L Mix, 4X 15 x 1 ml - PCR Water	
Storage	*In the dark at -20°C.*		

APPLICATIONS

- One-Step RT qPCR using crude lysed or impure samples
- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of total RNA, viral RNA, gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

BENEFITS

- Exceptional PCR-inhibitor tolerance in single-tube one-step RT qPCR
- Reduces the effects of common PCR inhibitors in clinical and environmental samples, food, animal and plant materials
- Concentrated mix with UDG/dUTP, Reverse Transcriptase and RNase Inhibitor for maximum template volume
- Amplifies both DNA and RNA templates, great for multiplexing, excellent performance on GC-rich templates

PRODUCT DETAILS

The SampleIN™ UDG 1Step RT qPCR Probe ROX L Mix has been specifically designed for use with crude lysates and impure templates. This 4X concentrated single-tube RT qPCR master mix with Hot Start Taq DNA Polymerase, Reverse Transcriptase, RNase Inhibitor, dNTPs, magnesium and optimized buffer delivers exceptional PCR inhibitor tolerance in direct one-step RT qPCR applications. It includes PCR enhancers and stabilizers and performs robustly even with unpurified saliva or fresh blood, tolerating chemicals carried over from purification as well as PCR inhibitors from blood, urine, plant and environmental samples (see list).
The mix can be used with all probe types and with a wide range of sample amounts, from very low-copy-number targets in diluted samples to abundant templates.
The mix contains all components required for a robust single-tube RT qPCR; only template, primers and probes need to be added. Its high concentration enables the use of maximal template volume as well as multiple primers and probes. The UDG version includes dUTP and UDG (uracil-DNA glycosylase), which degrades dUTP-containing contaminating DNA and prevents PCR carryover contamination.

Although the SampleIN™ UDG 1Step RT qPCR Probe ROX L Mix has been successfully tested with fresh blood and urine samples, template purification, or at least a fast lysis with the highQu SampleIN™ Lysis Set for PCR/qPCR (Cat.# DPL0101, DPL0105), is always recommended for more consistent results.
For crude or inhibitor-rich DNA templates, we offer the SampleIN™ UDG Direct qPCR Probe ROX L Mix (Cat.# DQP0401, DQP0405).
The SampleIN™ UDG 1Step RT qPCR Probe ROX L Mix contains ROX at low concentration; the version without ROX is available as SampleIN™ UDG 1Step RT qPCR Probe Mix (Cat.# DOS0301, DOS0305). If a high ROX concentration or more ROX flexibility is required, ROX for qPCR Mixes, 50 µM (Cat.# ROX0105) can be obtained separately and added directly to the qPCR mix.

TOLERATES COMMON INHIBITORS, SUCH AS:

- 5-7% crude saliva and crude blood in the reaction
- Chemicals left after NA extractions (guanidine, alcohols, SDS)
- Blood compounds (hematin, hemoglobin, hemin, immunoglobulins, heparin)
- Saliva and urine compounds (urea)
- Plant, soil samples (chlorophyll, humic and tannic acids, quercetin, polyphenols, cellulose)

PROTOCOL

- Protect RNA from RNase degradation: set up reactions in a separate, dedicated area and use DEPC-treated, nuclease-free labware and gloves. Check RNA quality on an agarose gel.
- Use primer design software for assay planning; amplicons of 80-200 bp (max. 400 bp) are recommended.
- Take typical measures to prevent PCR cross-contamination: keep your bench clean, wear gloves, use sterile tubes and filter pipette tips.
- Determine the best template amount for each new system with a dilution series; 1:10 and 1:100 dilutions are essential for crude lysates and unpurified blood, urine or saliva.
- Note that some crude sample components might cleave the probes; in such cases, template purification is necessary.
- Run reactions in triplicate; include a no-template control and a positive control with purified control DNA.
- Thaw and keep reagents on ice. Mix very well before use.
- Run an annealing temperature gradient for each new primer-template system to find the best annealing/extension temperature.
- Do not perform annealing/extension for longer than 30 seconds or at temperatures below 60 °C.
- For inhibitor-rich samples, an initial denaturation at 98°C for 5 min is recommended.
- Optimize new primer and probe concentrations within 500-1000 nM and 200-500 nM, respectively.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	~500-1000 nM final concentration
Forward Primer	~500-1000 nM final concentration
Specific Probe	~200-500 nM final concentration
RNA template cDNA Template *or* gDNA Template	2 pg-0.5 µg total RNA, >0.02 pg mRNA, >5 copies viral RNA <100 ng or 1 µg adjusted in 2-5 µl sample volume

- *Saliva can be used without lysis: dilute 1:10 in transport medium without guanidine and use 2-5 µl per 20 µl reaction.*
- *Blood can be used without lysis: dilute 1:10 in NaCl/EDTA and use 1-2 µl per 20 µl reaction.*
- *Use max. 2 µl of crude sample or cDNA reaction mixture per 20 µl reaction (up to 5 µl if diluted 1:10 or 1:100).*

PCR Water	to 15 µl
SampleIN™ UDG 1Step RT qPCR Probe ROX L Mix, 4X	5 µl

✓ Mix gently, avoid bubbles. Run the following program:

UDG step	25°C - 2 min (or ambient temperature)
RT step (RNA only)	45-55°C - 10 min (20 min for multiplex)
Initial denaturation	1 cycle: 95°C, 3 min (crude samples: 98°C, 5 min)
Denaturation	40-50 cycles: 95°C – 5-15 sec
Anneal./extension	40-50 cycles: 60-65°C – 5-20 sec

- *Replicates of the same crude sample may show different Cq values; 1-2 cycle delays are normal.*

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