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

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

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
DOS0201	200 r of 20 µl	1 ml - SampleIN™ 1Step RT qPCR Probe ROX L Mix, 4X 3 x 1 ml - PCR Water	The 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.
DOS0205	1000 r of 20 µl	5 x 1 ml - SampleIN™ 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 One-Step RT qPCR single-tube applications
- The unique composition reduces effects of common PCR inhibitors found in clinical, environmental samples, food matrices, animal, and plant materials
- Concentrated master mix containing Hot Start Tq Polymerase, Reverse Transcriptase and RNase Inhibitor for the use with maximum template volume
- Amplifies both DNA and RNA templates, great for multiplexing, excellent performance on GC-rich templates

PRODUCT DETAILS

The SampleIN™ 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 the Hot Start Taq DNA Polymerase, Reverse Transcriptase, RNase inhibitor, dNTPs, magnesium and optimized buffer delivers an exceptional PCR inhibitor tolerance in direct one-step qPCR applications. It includes PCR enhancers and stabilizers and is formulated to provide the robust performance with such common sample materials like unpurified saliva or fresh blood. The mix tolerates a range of common chemicals present in purified DNA templates such as guanidine, alcohols, SDS and similar, as well as common blood, urine and environmental natural sample-compounds known to inhibit PCR such as hemoglobin, immunoglobulins, heparin, urea, polyphenols, cellulose, humic and tannic acids and chlorophyl.
The mix can be used with all probe types and with a variety of sample amounts from a very low-copy number targets in diluted samples to abundant templates.
This 1Step RT qPCR Probe Mix contains all compounds required for robust single-tube RT qPCR reaction, and the only components to add are template, primers and probes. The high concentration of the master mix enables the use of maximal template volume as well as multiple probes and primers. The Mix is supplied with PCR Water.

Though the SampleIN™ 1Step RT qPCR Probe Mix has been successfully tested for the use with fresh blood and urine samples; for more consistent results, it is always recommended to purify the template, or at least to perform fast lysis using highQu SampleIN™ Lysis Set for PCR/qPCR.
For work with crude or inhibitor-rich DNA templates, we offer a SampleIN™ Direct qPCR Probe Mixes.
This SampleIN™ 1Step RT qPCR Probe Mix includes ROX at low concentration, the version without ROX is available as SampleIN™ 1Step RT qPCR Probe Mix. If high ROX concentration or more ROX flexibility is required, ROX for qPCR Mixes, 50 µM can be obtained separately and added directly into 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)
- Saliva and urine compounds (urea)
- Plant, soil samples (chlorophyl, humic, tannic acids, quercetin cellulose)

PROTOCOL

- Take care to prevent RNA from degradation by widely spread RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease free labware and gloves. Check RNA quality in agarose gel.
- Use special primer selection programs for good planning. Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross - contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Though the mix is tested for the use with fresh saliva, blood and urine samples, for consistent results, it is recommended to purify DNA, or to perform lysis using the SampleIN™ Lysis Set for PCR/qPCR or other lysis method.
- Initial template dilution series to determine the best template amount is recommended for each new system. 1:10 and 1:100 dilution series is especially important when working with crude lysed samples or pure samples such as fresh blood or urine or saliva.
- Note, that some crude sample components might cleave the probes, for such cases, DNA purification is necessary.
- Run reactions in triplets; include a no-template control and positive control with purified control DNA.
- Thaw and keep reagents on ice. Mix very well before use.
- Perform annealing temperature gradient for each new template-primer system. To evaluate best annealing/extension temperature.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.
- To inactivate and eliminate inhibitory effects, initial denaturation of 5 minutes at 98°C is recommended for certain inhibitor and contaminant-rich samples.
- Starting with new primers/probes, the optimal concentration of these shall be determined in a range of 500-1000 nM for primer and 200-500 nm for probes.

✓ Prepare a 20 µl reaction:

	500-1000 nM final concentration
	500-1000 nM final concentration
	200-500 nM final concentration
cDNA Template *or* gDNA Template	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, dilution in transport medium w/o guanidine is recommended. Use 2-5 µl of 1:10 diluted saliva in 20 µl reaction.

- *Blood samples can be used without lysis, 1:10 dilution in NaCl/EDTA is recommended. Use 1-2 µl of 1:10 diluted blood in 20 µl reaction.*
- *Reactions work with up to 5-7% volume of crude saliva or blood.*
- *For crude samples and cDNA reactions mixtures – do not use more than 2 µl of crude sample in 20 µl PCR reaction. For 1:10 or 1:100 diluted samples this volume can be maximized up to 5 µl.*

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

✓ Mix gently, avoid bubbles.

✓ Place into the instrument set like:

	-55°C - 10 min (20 min for multiplex)
(only for RNAs)	°C, 3 min (crude s.: 98°C - 5 m.)
	-50 cycles: 95°C – 5-15 sec
	-50 cycles: 60-65°C – 5-20 sec

Follow instrument instructions for melting curve analysis.

- *Note, that the same crude sample may show different Cq in replicates. 1-2 cycle delays are normal with crude samples.*

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