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

SampleIN™ UDG Direct qPCR Probe ROX L Mix, 4X

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
DQP0401	200 r of 20 µl	1 ml - SampleIN™ UDG Direct qPCR Probe ROX L Mix, 4X 3 x 1 ml - PCR Water	4X concentrated robust qPCR master mix with Hot Start Taq DNA Polymerase, dNTPs, magnesium, low ROX concentration and optimized buffer with enhancers and stabilizers. Includes dUTP and UDG (uracil-DNA glycosylase) to prevent PCR carryover contamination.
DQP0405	1000 r of 20 µl	5 x 1 ml - SampleIN™ UDG Direct qPCR Probe ROX L Mix, 4X 15 x 1 ml - PCR Water	
Storage	*In the dark at -20°C.*		

APPLICATIONS

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

BENEFITS

- Exceptional PCR-inhibitor tolerance in direct qPCR applications
- Reduces the effects of common PCR inhibitors in clinical and environmental samples, food, animal and plant materials
- Highly concentrated master mix with UDG and dUTP for use with maximum template volume, and multiple primers and probes
- Great for multiplexing, excellent performance on GC-rich templates

PRODUCT DETAILS

The SampleIN™ UDG Direct qPCR Probe ROX L Mix has been specifically designed for use with unpurified samples or crude lysates as templates. This 4X concentrated qPCR master mix with Hot Start Taq DNA Polymerase, dNTPs, magnesium and optimized buffer delivers exceptional PCR inhibitor tolerance in direct 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 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 Direct 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 RNA templates, we offer the SampleIN™ UDG 1Step RT qPCR Probe ROX L Mix (Cat.# DOS0401, DOS0405) with reverse transcriptase.
The SampleIN™ UDG Direct qPCR Probe ROX L Mix contains ROX at low concentration; the version without ROX is available as SampleIN™ UDG Direct qPCR Probe Mix (Cat.# DQP0301, DQP0305). 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

- Use primer design software for good assay planning.
- Work with amplicons of 80-200 bp (max. 400 bp).
- 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
cDNA Template *or* gDNA Template	<100 ng or 1 µg adjusted in 2-5 µl sample volume
PCR Water	to 15 µl
SampleIN™ UDG Direct qPCR Probe ROX L Mix, 4X	5 µl

- *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).*

✓ Mix gently, avoid bubbles.

✓ Place into the instrument and set as follows:

UDG step	25°C - 2 min (or ambient temperature)
Initial denaturation	1 cycle: 95°C - 3 min (up to 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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