

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

## Lyo-Ready SampleIN™ Direct qPCR Probe Mix, 4X

DQP010LR-1	200 r of 20 µl	1 ml - Lyo-Ready SampleIN™ Direct qPCR Probe Mix, 4X
DQP010LR-5	1000 r of 20 µl	5 ml - Lyo-Ready SampleIN™ Direct qPCR Probe Mix, 4X
DQP010LR-50	10000 r of 20 µl	50 ml - Lyo-Ready SampleIN™ Direct qPCR Probe Mix, 4X

Storage In the dark at -20°C.

Glycerol-free 4X concentrated robust Direct qPCR Probe Mix contains Hot Start Taq, dNTPs, magnesium, buffer with excipients and is formulated for lyophilization of inhibitor tolerant DNA detection assays that are stable at ambient temperatures.

### APPLICATIONS

- Development of lyophilized assays for pathogen DNA detection
- Viral, bacterial DNA detection in diluted and inhibitor-rich samples
- Probe-based assays: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of low copy number genes
- qPCR using crude lysed or even unpurified samples

### PRODUCT DETAILS

The SampleIN™ Direct qPCR Probe Mix has been specifically designed for use with crude lysates and impure templates. This 4X concentrated qPCR master mix with the Hot Start Taq DNA Polymerase, dNTPs, magnesium, and optimized buffer delivers an exceptional PCR inhibitor tolerance in direct 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 chlorophyll. This Direct qPCR Probe Mix contains all compounds required for robust qPCR reaction, and the only components to add are template, primers and probes. The Lyo-ready version of the mix allows for the same sensitivity of DNA target detection with a help of lyophilized kits that can be stored and shipped without cooling.

### PROTOCOL FOR REACTION SET UP AND QPCR CYCLING

- Use special primer selection programs, 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.
- 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 SampleIN™ Lysis Set for PCR/qPCR.
- 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.
- Some 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.
- Lyo-Ready 4X concentrated Mix includes all essential components for qPCR. You only need to add target, primers, specific Probe and optionally, ROX. After adding all components, the mix is ready to be lyophilized, or to be tested in qPCR as described below, preferably on the same day.
- Keep the mixed solution cold at +4°C up to qPCR cycling or up to lyophilization.

#### For wet testing, 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	<100 ng or
gDNA Template	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.
- 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.

PCR Water	to 15 µl
SampleIN™ Direct qPCR Probe Mix, 4X	5 µl

- Mix gently, avoid bubbles. Place into the instrument set like:
- |                      |  |
|----------------------|--|
| 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           |

Follow instrument instructions for melting curve analysis.

Note, that the same crude sample may show different C<sub>q</sub> in replicates. 1-2 cycle delays are normal with crude samples.

### RECOMMENDATIONS FOR LYOPHILIZATION

- After establishing the right primer-probe and template concentration, as well as the optimal cycling (annealing) conditions, go on with lyophilization. To proceed, add Primers (0.5-1 µM final conc.) and Probe (200-500 nM final conc.) and dilute the final mix to a 1X concentration with nuclease-free water. ROX might be added prior to lyophilization.

### BENEFITS

- Exceptional PCR-inhibitor tolerance in direct qPCR applications with clinical, environmental samples, food matrices, animal, and plant materials
- Can be used under the ultrafast cycling conditions; outperforms many competitors
- Detects <5 copies, ideal for DNA targets in crude samples of saliva or blood
- Glycerol-free, lyo-ready formulation, with an excellent performance after the reconstitution, and 12 months ambient temperature stability of lyophilized mix

The Lyo-ready mix includes all the same components plus excipients in optimized buffer, and is ready for lyophilization. After the reconstitution of the assays, the mix performs same efficient as before the lyophilization. The mix is suitable for preparing lyophilization beads or cakes in both plastic or glass vials, however, for each lyophilization workflow the conditions have to be optimized. Though the SampleIN™ Direct 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 f. e. highQu SampleIN™ Lysis Set for PCR/qPCR.

#### TOLERATES COMMON INHIBITORS, SUCH AS:

- <3% untreated saliva and <6% blood (in EDTA) the reaction
- Chemicals left after NA extractions (guanidine, alcohols, SDS)
- Blood compounds (hematin, hemoglobin, hemin, immunoglobulins)
- Saliva and urine compounds (urea)
- Plant, soil samples (chlorophyll, humic, tannic acids, quercetin cellulose)
- For each new assay, optimal lyophilization parameters differ, and must be optimized.
- Onset T<sub>g</sub> (glass transition) temperature is 74.6°C.

#### Example of lyophilization conditions in a glass vial:

Step	Temp. °C	Time min.	Pressure	Explanation
Initialization 0	+4	NA	1.01325 bar	Load
Initialization 1	+5	10	1.01325 bar	Hold
Initialization 2	-50	110-220	1.01325 bar	Ramp 0.3-0.5°C/min
Initialization 3	-50	180	1.01325 bar	Hold
Primary drying 4	-45	10-20	0.03 mbar	Ramp 0.3-0.5°C/min
Primary drying 5	-45	5500	0.03 mbar	Hold
Secondary drying 6	+20 to +25	150	0.03 mbar	Ramp 0.3-0.5°C/min
Secondary drying 7	+20 to +25	600	0.03 mbar	Hold
Termination 8	+20 to +25	NA	0.507 bar	Backfill N <sub>2</sub>
Termination 9	+20 to +25	NA	0.507 bar	Stopper
Termination 10	+20 to +25	NA	1.01325 bar	Aerate

#### Example of lyophilization conditions in plastic PCR tubes or 96-well plates:

Initialization 0	-20	NA	1.01325 bar	Load
Initialization 1	-20	10	1.01325 bar	Hold
Initialization 2	-50	110-220	1.01325 bar	Ramp 0.165°C/min
Initialization 3	-50	60	1.01325 bar	Hold
Primary drying 4	-45	10-20	0.03 mbar	Ramp 0.5°C/min
Primary drying 5	-45	900	0.03 mbar	Hold
Secondary drying 6	+20 to +25	130	0.03 mbar	Ramp 0.5°C/min
Secondary drying 7	+20 to +25	250	0.03 mbar	Hold
Termination 8	+20 to +25	NA	0.507 bar	Backfill N <sub>2</sub>
Termination 9	+20 to +25	NA	1.01325 bar	Aerate

#### Example of lyophilization conditions for beads:

Initialization 0	-50	NA	1.01325 bar	Load
Initialization 1	-50	110-220	1.01325 bar	Hold
Initialization 2	-50	30	0.06 bar	Hold
Primary drying 3	-45	10	0.06 bar	Ramp 0.5°C/min
Primary drying 4	-45	3500	0.06 bar	Hold
Secondary drying 5	+20 to +25	130	0.06 bar	Ramp 0.5°C/min
Secondary drying 6	+20 to +25	360	0.06 bar	Hold
Termination 7	+20 to +25	NA	0.507 bar	Backfill N <sub>2</sub>
Termination 8	+20 to +25	NA	1.01325 bar	Aerate

- ✓ Reconstitution of beads and tubes: add test sample with PCR water to ~20 µl, mix slowly, and run the cycling program.
- ✓ Reconstitution of cakes: add PCR water to a 2X mix concentration, mix slowly, distribute the mix into the single tubes and bring the volume with water and test sample to a 1X concentration; mix and run the cycling program.

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