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

Lyo-Ready ORA™ qPCR Probe Mix, 4X

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP070LR-1	200 r of 20 µl	1 ml – Lyo-Ready ORA™ qPCR Probe Mix, 4X	Glycerol-free, Lyo-Ready qPCR Probe Mix, 4X contains Hot Start Taq, dNTPs, magnesium (4.5 mM final 1X conc.), buffer with excipients.
QPP070LR-10	2000 r of 20 µl	2 x 5 ml – Lyo-Ready ORA™ qPCR Probe Mix, 4X	
QPP070LR-50	10 000 r of 20 µl	50 ml – Lyo-Ready ORA™ qPCR Probe Mix, 4X	
QPP070LR-500	100 000 r of 20 µl	500 ml – Lyo-Ready ORA™ qPCR Probe Mix, 4X	
Storage	*In the dark at -20°C.*		

APPLICATIONS

- Development of lyophilized assays for pathogen detection
- Viral DNA/cDNA detection in diluted low copy number samples
- qPCR or combined RT 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

- Glycerol-free, lyophilization-ready formulation of robust 4X qPCR mix for high sample volume input
- Universal mix - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex and multiplexing, high throughput
- Rapid extension, early Ct

PRODUCT DETAILS

The glycerol-free Lyo-Ready ORA™ qPCR Probe Mix, 4X is designed for a sensitive detection of specific nucleic acids in diluted high-volume samples. The lyophilization ready format allows production of ambient temperature stable diagnostic qPCR or RT qPCR kits. highQu qPCR master mixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization.
The low-glycerol Lyo-ready version of the mix allows for the same sensitivity of DNA detection with a help of lyophilized reagents that can be conveniently stored and shipped without cooling.

TECHNICAL DATA

The 4X concentrated glycerol-free, Lyo-Ready ORA™ qPCR Probe Mix contains Hot Start Taq, dNTPs, magnesium, and buffer with excipients ready for lyophilization.

The mix is suitable for preparing lyophilization beads or cakes in both plastic or glass vials.
For each lyophilization workflow the conditions have to be optimized.

To distribute all components of the solution evenly, the mix shall be mixed well after each thawing.

PROTOCOL

1. Thaw and keep reagents on ice. It is very important to mix them very well before use and to spin down all the drops!
2. You can check the performance of the liquid Lyo-Ready ORA™ qPCR Probe Mix, 4X by setting up the qPCR reaction as described in the example below. Alternatively, or afterwards go to step 3 to proceed with lyophilization.

   ✓ Use 1-5 microliters of template or swab extract for 20 µl reaction.
   ✓ Optimization (annealing temperature gradient) in a range 56 - 66°C is recommended to determine optimal annealing/extension temperature.

   ✓ Prepare a 20 µl reaction:

Lyo-Ready ORA™ qPCR Probe Mix, 4X	5 µl
Reverse & Forward Primer	0.5 – 1 µM final conc. each
Specific Probe	130 – 500 nM final conc.
Template (DNA or crude sample)	1 – 5 µl 100 ng cDNA/<1 µg gDNA
PCR-grade Water	to 20 µl

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

Taq activation	1 cycle: 95°C - 2 min
Denaturation	40-50 cycles: 95°C – 10-15 sec.
Annealing/extension	40-50 cycles: 58°C (56-66°C) – 20-30 sec.

   ✓ Follow instrument instructions for melting curve analysis.

3. To proceed with lyophilization, add Primers (0.5-1 µM final conc.) and Probe (130–500 nM final conc.).
4. For optimal lyophilization outcome, we recommend to dilute the final total reagent mix to a 1X - 2X concentration with nuclease free water.
5. Optionally, required ROX amount might be added prior to lyophilization. It shall not interfere with any of further processes.
6. Important temperature parameters:
   Onset T_c (collapsing) is -35.1°C / Onset T_g (glass transition) is 68.9°C
   Mid-point T_g is 75.1°C / End-point T_g is 81.3°C
7. The 0.5 ml of the mix has been tested for lyophilization in 2 ml glass vials. Optimization is required for other vials and instruments. For example, plastic PCR tubes will need less drying time.

   Example of lyophilization conditions:

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_2
Termination 9	+20 to +25	NA	0.507 bar	Stopper
Termination 10	+20 to +25	NA	1.01325 bar	Aerate

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