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

AirDry 4X 1Step RT qPCR Probe Mix

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP080AD-1	200 r of 20 µl	1 ml – AirDry 4X 1Step RT qPCR Probe Mix	Glycerol-free AirDry 4X concentrated 1Step RT qPCR Probe Mix contains Hot Start Taq, dNTPs, magnesium, buffer with excipients and the blend of modified MMuLV RT and a highly efficient RNase Inhibitor formulated for air-drying of RNA/DNA detection assays.
QOP080AD-10	2000 r of 20 µl	2 x 5 ml – AirDry 4X 1Step RT qPCR Probe Mix	
QOP080AD-50	10 000 r of 20 µl	50 ml – AirDry 4X 1Step RT qPCR Probe Mix	
QOP080AD-500	100 000 r of 20 µl	500 ml – AirDry 4X 1Step RT qPCR Probe Mix	
Storage	*In the dark at -20°C.*		

APPLICATIONS

- Development of air-dried assays for virus/pathogen RNA/DNA or other set target sequence detection
- Target RNA/DNA detection in highly diluted samples
- RT qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of mRNA, total RNA, viral RNA, low copy genes

BENEFITS

- Air dryable, single-tube format 1Step RT qPCR Mix
- qPCR mix blended with Reverse transcriptase and RNase Inhibitor
- Fast, oven drying procedure for ambient storage of diagnostic kits
- Just add primers and probes, oven-dry in minutes, and the end user will reconstitute it with the aqueous sample solution for testing
- Ideal for multiplex reactions, universal for RNA or DNA detection

PRODUCT DETAILS

The AirDry 4X 1Step RT qPCR Probe Mix is designed for development of room temperature stable assays which ensure a sensitive detection of specific RNAs and DNAs in diluted low-copy number samples. This 4X Rt qPCR mix with Hot Start Taq, dNTPs, magnesium, thermostable Reverse Transcriptase and RNase Inhibitor in an optimal buffer allows for a single step, one tube RT qPCR reactions ensuring high detection sensitivity. The air dryable formulation contains stabilizers and excipients to ensure same great performance of the mix both before the drying and after the drying, room temperature shipment and reconstitution.

The mix is therefore an excellent choice to develop RNA or DNA multiplex target detection kits that can be shipped and stored at ambient temperature. Multiplex RNA (or DNA) target detection systems can be created just by adding appropriate primers and probes (TaqMan®, Molecular Beacons, Scorpions™) and primers. ROX can be added if required. This prepared and in wet-form optimized detection mix can be air-dried in a convection oven within 60-90 minutes, closed, and shipped at ambient temperature. The end user just adds water-diluted test sample into the gel-like dried mix and after short reconstitution by slow shaking, detects pathogens or other DNA/RNA targets in a fast qPCR workflow with the highest detection sensitivity.

PROTOCOL FOR REACTION SET UP AND QPCR CYCLING

1. Thaw and keep reagents on ice. Mix them very well before use.
2. AirDry 4X concentrated 1Step RT qPCR Probe Mix includes all essential components for cDNA synthesis and qPCR: reverse transcriptase, RNase inhibitor, Hot Start Taq, dNTPs, magnesium, buffer with excipients. You only need to add target specific Probe and optionally, ROX.
3. After adding all the components, the mix is ready to be dried in the convection oven immediately, or to be tested in qPCR cycler as described below, preferably on the same day.
4. Keep the mixed solution cold at +4°C up to qPCR cycling (step5)
5. For wet testing, prepare a 20 µl reaction:

AirDry 4X 1Step RT qPCR Probe Mix	5 µl
Minimal volume of Primers	0.5 – 1 µM final conc. each
Minimal volume of Probe	130 – 500 nM final conc.
Optionally, add ROX, mix and move to step 6 or proceed with qPCR:	
Template (RNA/DNA/crude sample)	To 20 µl (in PCR Water)

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

Reverse Transcription (for RNA templates)	1 cycle: 52°C (45-55°C) for 5-10 (to 20) min
RT inactivation/PCR activation	1 cycle: 95°C - 3 min
Denaturation	50 cycles: 95°C - 15 sec
Annealing/extension	50 cycles: 58°C (55-65°C) - 30 sec.

✓ Follow instrument instructions for melting curve analysis.

Notes for cycling conditions:

✓ 5 min. are enough for reverse transcription at 45-55°C. For multiplexing up to 6-plex, prolong RT step to 10-20 min. Skip RT step for DNA templates.
✓ Do not perform annealing/extension for more than 30 seconds. Use 58 °C temperature for this step. Optimize in a range 56 - 65°C.

AIR DRYING RECOMMENDATIONS

6. To proceed with air-drying procedure, after adding primers, probes and (optional) ROX, mix the master mix well, and dry it:

✓ Pipet 5-6 µl of the final RT qPCR mix into the 96-well plate wells.
✓ Put the plate into the convection oven set at 40°C.
✓ Let the water evaporate (air-dry) for 60-90 minutes.
✓ Control the drying efficiency using *LOD* (loss on drying) formula:

LOD=(W2-W3)/W2-W1)x100
W1 – weight of the empty plastic tube/plate
W2 - weight of the plastic tube/plate filled with the wet mix
W3 - weight of the plastic tube/plate after drying the mix
Good LOD is about ~70, what gives the gel with ~<10% of residual water.

✓ Use sachet silica to keep packed plates dry.
✓ Dried plates can be stored and shipped at room temperature with an approximate shelf life of 12 months (depends on your assay).
✓ The user will have to dissolve the mix in ~18-20 µl RNA/DNA sample solution prepared with the PCR grade water, mix slowly for complete reconstitution and run the RT qPCR or PCR cycling protocol as you will recommend optimized for your assay.

Notes for air-drying conditions:

✓ Drying conditions have to be evaluated experimentally for each oven/test/volume. The time of the drying will vary depending on the oven, the volume, and the temperature.
✓ 40°C is recommended temperature for drying and keeping the unchanged activity of all components. 50°C is maximum possible, because the reverse transcriptase included in the mix is the sensitive component.
✓ However, in case you use the mix for DNA target detection only and do not need functional RT, you may use 80°C for drying the mix in 20 min.

Example of air-drying conditions to variate when evaluating the best ones:

Step	*Volume*	*Temp. °C*	*Time min.*	*Comment*
Air drying 1	*5 µl*	*40*	*70*	*for RNA detection*
Air drying 2	*5 µl*	*45*	*60*	*for RNA detection*
Air drying 3	*6 µl*	*40*	*80*	*for RNA detection*
Air drying 4	*6 µl*	*45*	*70*	*for RNA detection*
Air drying 5	*5 µl*	*50*	*60*	*for DNA detection*
Air drying 6	*5 µl*	*60*	*50*	*for DNA detection*
Air drying 7	*6 µl*	*60*	*60*	*for DNA detection*
Air drying 8	*6 µl*	*70*	*50*	*for DNA detection*

IN VITRO RESEARCH USE ONLY

ORDERING
T: +49 7250 33 13 401
F: +49 7250 33 11 413
order@highQu.com
www.highQu.com

SALES
T: +49 7250 33 13 401
F: +49 7250 33 11 413
sales@highQu.com

TECHNICAL SUPPORT
T: +49 7250 33 13 401
F: +49 7250 33 11 413
tech-support@highQu.com