

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

AirDry SampleIN™ 1Step RT qPCR Probe Mix

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
DOS010AD-1	200 r of 20 µl	1 ml - AirDry SampleIN™ 1Step RT qPCR Probe Mix, 4X	Nearly glycerol-free 4X concentrated robust 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 inhibitor resistant/tolerant RNA/DNA detection assays stable at ambient temperatures.
DOS010AD-5	1000 r of 20 µl	5 ml - AirDry SampleIN™ 1Step RT qPCR Probe Mix, 4X	
DOS010AD-50	10 000 r of 20 µl	50 ml - AirDry SampleIN™ 1Step RT qPCR Probe Mix, 4X	

Storage In the dark at -20°C.

APPLICATIONS

- Production of air-dried ambient temperature stable kits
- RTqPCR using crude lysed or even unpurified samples
- RTqPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of RNA, cDNA, viral NA, low copy number genes

PRODUCT DETAILS

The AirDry SampleIN™ 1Step RT qPCR Probe Mix, 4X has been specifically designed for use with unpurified samples or crude lysates as templates. This 4X concentrated master mix with the Hot Start Taq DNA Polymerase, dNTPs, magnesium, and optimized buffer delivers an exceptional PCR inhibitor tolerance in direct RT qPCR applications. It includes PCR enhancers and stabilizers and is formulated to provide 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 Mix contains all compounds required for robust RT qPCR reaction, and the only components to add are template, primers and probes.

PROTOCOL FOR REACTION SET UP AND QPCR CYCLING

- Take care to prevent RNA from degradation by widely spread RNases.
- Use special primer selection programs for good planning. Work with amplicons in a range of 80-200, max 400 bp.
- 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, NA 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 temperature. 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 60 °C temperature for this step (range 58 - 65°C)
- The optimal concentration of primers/probes shall be determined in a range of 500-1000 nM for primer; 200-500 nM for probes.
- AirDry 4X concentrated 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 (RNA or DNA) and optionally, ROX.
- After adding all components, the mix is ready to be dried in the convection oven, 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
- **For wet testing, prepare a 20 µl reaction:**

1Step RT qPCR Probe Mix, 4X	5 µl
Minimal volume of Primers	0.4 – 1 µM final conc. each
Minimal volume of Probe	200 – 500 nM final conc.
Optionally, add ROX, mix.	
Template (RNA/DNA)	To 20 µl (in PCR Water)
<ul style="list-style-type: none"> • Mix gently, avoid bubbles. • Place into the instrument set like: 	
Reverse Transcription (only for RNA templates)	1 cycle: 52°C (45-55°C) for 5-10 min (to 20 min for multiplex)
RT inactivation/PCR activation (for all DNA/RNA templates)	1 cycle: 95°C, 3 min (crude samples: 98°C - 5 min)
Denaturation	40-50 cycles: 95°C - 5-15 sec
Annealing/extension	40-50 cycles: 60°C (58-65°C) - 5-20 sec.

- ✓ Follow instrument instructions for melting curve analysis.

BENEFITS

- Exceptional PCR-inhibitor tolerance in direct 1Step RT qPCR applications
- 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 detection of both RNA and DNA targets in crude samples of saliva or blood

The air dryable formulation ensures same great performance of the mix after the drying, room temperature shipment, and reconstitution. The mix is an excellent choice to develop multiplex target detection kits that can be shipped and stored at ambient temperature. The prepared and in wet-form optimized RT qPCR detection mix with primers and probes 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 shaking, detects pathogens or other NA targets in a fast RT qPCR workflow with the highest sensitivity. Though the SampleIN™ Mixes have been successfully tested for the use with fresh blood and urine samples; for consistent results, it is recommended to purify the template, or to perform lysis using SampleIN™ Lysis Set for PCR/qPCR.

TOLERATES COMMON INHIBITORS, SUCH AS:

- 5 % crude saliva and crude blood in the reaction
- Chemicals left after NA extractions (guanidine, alcohols, SDS)
- Blood compounds (hematin, hemoglobin, hemin, immunoglobulins)
- Plant, soil samples (chlorophyll, humic, tannic acids, quercetin cellulose)

AIR DRYING RECOMMENDATIONS

To proceed with air-drying procedure, after adding primers, probes, mix well, and dry it:

- ✓ Pipet 5-6 µl of the final 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:

$$LOD = (W2 - W3) / (W2 - W1) \times 100$$

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, to mix slowly for complete reconstitution and run the cycling protocol as you will recommend optimized for your assay.

Notes for air-drying conditions:

- ✓ Drying conditions must 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 is sensitive.
- ✓ 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