Instructions for Use
Life Science Kits & Assays

innuDRY qRT-PCR Mix Probe

Innuscreen GmbH

1 Product specifications

The innuDRY qRT-PCR Mix Probe is designed for efficient cDNA synthesis and subsequent qPCR in a single tube. It can be used to quantify any RNA template including mRNA, total RNA and viral sequences. Extremely low copy number targets can be detected specifically with high efficiency. The Mix can be used for the probe-detection technology including TaqMan and Rehybridization Probe System.

The freeze-dried formulation of 5x MasterMix allows shipment and storage without cooling.

Only template, primers and probe need to be added to the reaction and the final volume should be filled up with PCR-grade water.

2 Quality data and unit definition

Activity and stability tested by low copy PCR, human DNA contamination and activity of DNase and RNase are not detected.

Use 1pg to 1µg total RNA per reaction, use minimum 0.01pg mRNA per reaction.

3 Product and order number

Name	Amount	Order-no.
innuDRY qRT-PCR Mix Probe	100 rxn	845-RT-9000100
innuDRY qRT-PCR Mix Probe	200 rxn	845-RT-9000200

4 Storage conditions

The innuDRY qRT-PCR Mix Probe is delivered at room temperature.
It is recommended to store the lyophilized Mix at +4 to +8°C in a fridge.
After resolving store at -22 to -18 °C in a freezer with constant temperature conditions.
When stored as recommended, the Mix is stable until the expiration date printed on the label on the kit box.

5 Delivered components

Component	Description	Amount
innuDRY qRT-PCR Mix Probe 	Concentration if dissolved in Reconstitution Buffer Probe: 5x	Lyophilized pellet
Reconstitution Buffer Probe 	Reaction Buffer	400 µl

6 Safety precautions

The assay shall only be handled by educated personal in a laboratory environment. The compliance with the specified procedure is absolutely mandatory when performing this assay.

Reagents should be stored in their original containers at the indicated temperatures. Do not replace individual components with those from different batches or test assays. Note the indicated expiration dates.

Do not eat, drink or smoke while performing the assay.

Wear protective clothing and safety gloves.

All samples and test materials should be handled and disposed of as infectious material, in accordance with regulatory requirements.

Reagent containers that have not come in contact with potentially infectious material may be disposed of along with ordinary laboratory waste.

Store the reagents used for performing PCR separately from DNA/RNA templates and amplification products.

7 Reagent preparation

Steps before using the innuDRY qRT-PCR Mix Probe:

1. Open the tube caps and pipette 400 µl of Reconstitution Buffer Probe into the lyophilized innuDRY qRT-PCR Mix Probe tube.
2. Close the tube cap and gently mix by vortexing to get a homogenous solution. Briefly centrifuge for a few seconds to collect the mixture at the bottom of the tube.
3. Store the ready-to-use qRT-PCR Mix Probe

   at -22 to -18 °C.

Setup of the PCR

- Gently vortex and briefly centrifuge the Mix after thawing
- Mix following components for 1 reaction

Reagent	Volume (1 rxn)
5x innuDRY qRT-PCR Mix Probe	4 µl
Forward Primer	0.2 - 1 µM
Reverse Primer	0.2 - 1 µM
Probe	0.2 - 1 µM
Template RNA	1 - 100 ng/µl (max. 1 µg)
PCR-grade H_2O	add to a final vol. of 20 µl
Total volume	20 µl

- After pipetting mix the components of the reaction mix by gently vortexing and briefly centrifugation for a few seconds to collect the mixture at the bottom of the tube.
- Reserve plate positions for positive (control RNA) and negative (water or buffer) controls.
- When preparing mixes, always calculate the volume according to the number of reactions that you need plus one extra.

Note: Reaction conditions (incubation temperatures and times, concentrations of template RNA, primers) depend on template and primers used.

8 PCR conditions

Step	Cycles	Profile	Temperature	Retention time
1	1	Reverse Transcription	50 °C	10 min
2	1	Initial Denaturation	95 °C	3 min
3	40	Denaturation	95 °C	10 - 30 sec
		Annealing	60 - 68 °C	30 - 60 sec

Note: Annealing temperature should be 2 - 6 °C lower than melting temperature of primer.

9 Hints and Notes

- For efficient amplification under fast cycling conditions we recommend amplicon lengths between 80 bp and 200 bp.
- The shorter the amplicon length the faster the reaction can be cycled.
- Amplicon lengths should not exceed 400 bp.
- qRT-PCR is a very sensitive amplification reaction. Therefore, care should be taken to eliminate the possibility of contamination with any foreign RNA/DNA templates or PCR products.

Publication No.: HB_RT-9000_e_210908

Manufacturer:
IST Innuscreen GmbH
Robert-Rössle-Strasse 10
13125 Berlin · Germany

Telefon +49 30 94893380
Telefax +49 30 94893381
Info.innu@ist-ag.com