Instructions for Use
Life Science Kits & Assays

innuDRY qPCR MasterMix Probe

analytikjena
An Endress+Hauser Company

1 Product specifications

The innuDRY qPCR MasterMix Probe has been developed for fast, highly reproducible real-time PCR and has been validated on commonly used real-time PCR instruments. It contains all reagents required for real-time PCR.

The 2x MasterMix has been formulated for use with probe-detection technology including TaqMan and Rehybridization Probe System. A combination of the latest advances in buffer chemistry and PCR enhancers together with an aptamere blocked hot-start DNA polymerase ensures fast, highly-specific and ultra-sensitive real-time PCR results.

Only the template, probes and primers need to be added to the reaction and the final volume should be filled up with PCR-grade water. The Mix does not include any reference dyes.

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. Polymerization activity at 25 °C is not detected.

One unit of enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides (dNTP's) into a polynucleotide fraction in30 minutes at 70 °C.

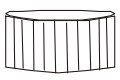
3 Product and order number

Name	Amount	Order-no.
innuDRY qPCR MasterMix Probe	100 rxn	845-AS-1900100
innuDRY qPCR MasterMix Probe	200 rxn	845-AS-1900200

4 Storage conditions

The innuDRY qPCR MasterMix Probe is delivered at room temperature.
It is recommended to store the lyophilized MasterMix 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 MasterMix is stable until the expiration date printed on the label on the kit box.

5 Delivered components

Component	Description	Amount
innuDRY qPCR MasterMix Probe 	Concentration if dissolved in Resuspension Buffer: 2x Nucleotides (dATP, dCTP, dGTP, dTTP): 0.4 mM each Taq DNA polymerase:1 Unit per 20 µl PCR reaction volume	Lyophilized pellet
Resuspension Buffer Probe 	Reaction Buffer incl. an appropriate amount of $MgCl_2$	1100 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 templates and amplification products.

7 Reagent preparation

Steps before using the innuDRY qPCR MasterMix Probe:

1. Open the tube caps and pipette 1.0 ml of Resuspension Buffer Probe into the lyophilized innuDRY qPCR MasterMix 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 qPCR MasterMix Probe

   at -22 to -18 °C.

Setup of the PCR

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

Reagent	Volume (1 rxn)
2x innuDRY qPCR MasterMix Probe	10 µl
Forward Primer	0.2 - 1 µM
Reverse Primer	0.2 - 1 µM
Probe	0.2 - 1 µM
Template DNA	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 DNA) 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 DNA, primers) depend on template and primers used.

8 PCR conditions

Step	Cycles	Profile	Temperature	Retention time
1	1	Initial denaturation	95 °C	120 s
2	40	Denaturation	95 °C	10 - 30 sec
		Annealing	50 - 68 °C	30 - 60 sec

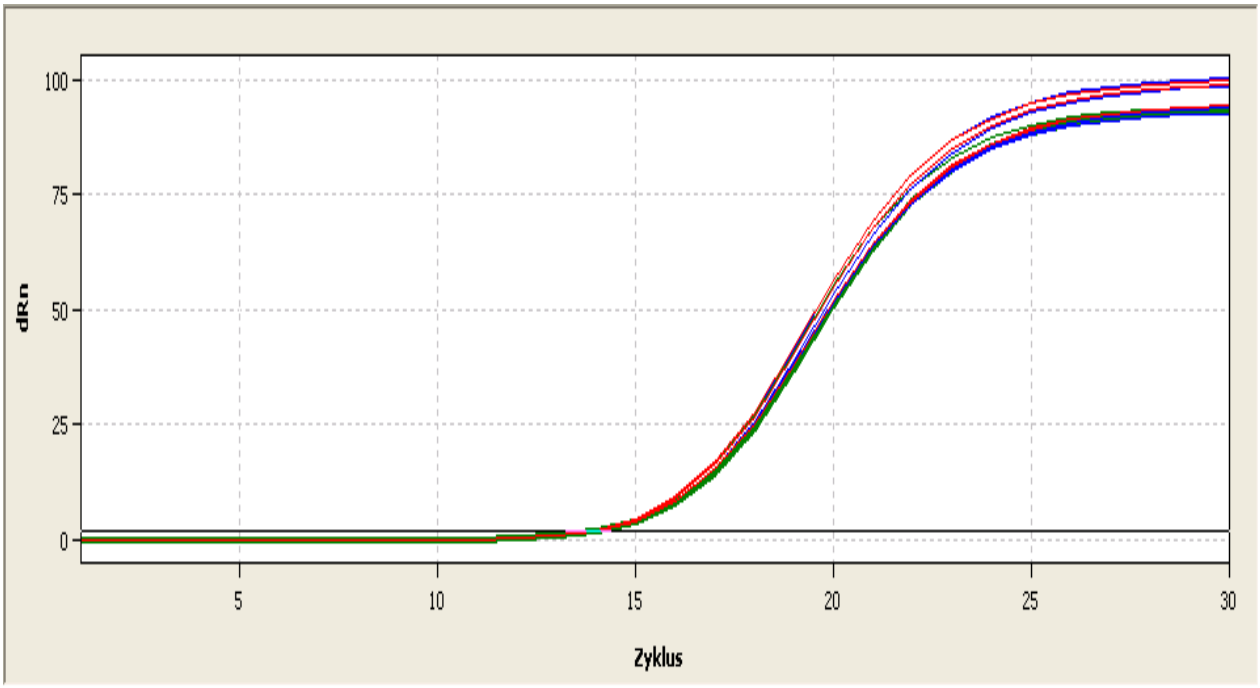
Note: Annealing temperature should be 2 - 6 °C lower than melting temperature of primer.

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.
- qPCR is a very sensitive DNA amplification reaction, therefore care should be taken to eliminate the possibility of contamination with any foreign DNA templates or PCR products.

10 Application examples

Detection of the amplified products of the Cytochrom b gene of human DNA by FAM labelled probe using the fresh pipetted PCR chemicals (red), Liquid MasterMix (green) and innuDRY qPCR MasterMix Probe (blue).



11 Related products

Product	Order Number
innuMIX qPCR MasterMix SyGreen	845-AS-1300100
innuMIX Green PCR MasterMix	845-AS-1400100
innuMIX Standard PCR MasterMix	845-AS-1700100
innuDRY Standard PCR MasterMix	845-AS-2100100
innuMIX qPCR MasterMix Probe	845-AS-1200100

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