Instructions for Use
Life Science Kits & Assays

innuMIX qPCR MasterMix Probe

Innuscreen GmbH

1 Product specifications

The innuMIX qPCR MasterMix Probe is designed for fast and highly reproducible real-time PCR (qPCR). It has been validated on commonly used qPCR instruments and contains all necessary reagents for the reaction, only template DNA, primers, and probes need to be added. The final reaction volume should be adjusted with PCR-grade water.

Key Features and Benefits:

Ready-to-use 2x MasterMix

Optimized for probe-based detection systems, including:

- TaqMan®
- Rehybridization Probe Systems

Advanced formulation

Combines:

- Innovative buffer chemistry
- PCR enhancers
- Aptamer-blocked hot-start DNA polymerase
- High quality dNTP's

→ Ensures fast, highly specific, and ultra-sensitive qPCR results

Versatile applications

Suitable for:

- Multiplex real-time PCR
- Endpoint PCR
- Amplification of long DNA fragments (e.g., for nanopore sequencing)

Note:

The MasterMix does not contain 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 in 30 minutes at 70 °C.

3 Product and order number

Name	Amount	Order-no.
innuMIX qPCR MasterMix Probe	15 rxn	845-AS-1200015
innuMIX qPCR MasterMix Probe	100 rxn	845-AS-1200100
innuMIX qPCR MasterMix Probe	200 rxn	845-AS-1200200
innuMIX qPCR MasterMix Probe	1000 rxn	845-AS-1201000

4 Storage conditions

innuMIX qPCR MasterMix Probe is delivered at room temperature. Store the MasterMix 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 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.

6 Reagent preparation

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

Reagent	Volume/ amount (1 rxn)
2x innuMIX qPCR MasterMix Probe	10 µl
Forward Primer	3 - 5 µM
Reverse Primer	3 - 5 µM
Probe (depending on application)	2,5 - 5 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.

7 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
		Elongation (Optionally)	72°C	60 sec/1 kb

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

8 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 for RealTime PCR should not exceed 400 bp.
- Amplification of longer fragments requires an additional extension step at 72°C in each cycle.
- The optimal $MgCl_2$ concentration for most RealTime PCR reactions is between 3 and 6 mM. The final concentration of the master mix in the PCR reaction is 5 mM. If necessary, the concentration can be optimized using a $MgCl_2$ solution.
- 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.

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