Instructions for Use
Life Science Kits & Assays

innuMIX (q)PCR MasterMix Economy

Innuscreen GmbH

1 Product specifications

The innuMIX (q)PCR MasterMix Economy is designed for routine and reliable real-time PCR applications. It has been validated on commonly used real-time PCR instruments that do not require ROX normalization. It contains all reagents required for PCR.

The innuMIX (q)PCR MasterMix Economy is available in extra-large packaging, which helps reduce both product costs and environmental impact.

This master mix can also be used for endpoint PCR.

Only the DNA template primers and possibly probes need to be added to the reaction mix. The final volume should be adjusted 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 in 30 minutes at 70 °C.

3 Product and order number

Name	Amount	Order-no.
innuMIX (q)PCR MasterMix Economy	15 rxn	845-AS-1330015
innuMIX (q)PCR MasterMix Economy	500 rxn	845-AS-1330500
innuMIX (q)PCR MasterMix Economy	1000 rxn	845-AS-1331000
innuMIX (q)PCR MasterMix Economy	2000 rxn	845-AS-1332000

4 Storage conditions

innuMIX (q)PCR MasterMix Economy 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 Economy	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 3,5 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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