Instructions for Use
Life Science Kits & Assays

innuMIX qPCR DSGreen Standard

analytikjena
An Endress+Hauser Company

1 Product specifications

The innuMIX qPCR DSGreen Standard has been developed for fast, highly reproducible real-time PCR and has been validated on commonly used real-time PCR instruments with *No-ROX* requirements. It contains all reagents required for real-time PCR and is designed to achieve excellent results in reaction efficiency and slope.

The MasterMix can be used to detect any DNA template even in difficult crude samples (like blood samples and bisulfite treated DNA).

The proprietary technology prevents formation of primer dimers and non-specific products leading to improved reaction sensitivity and specificity. Only the template and primers 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. 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

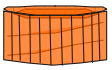
Name	Amount	Order-no.
innuMIX qPCR DSGreen Standard	100 rxn	845-AS-1320100
innuMIX qPCR DSGreen Standard	200 rxn	845-AS-1320200
innuMIX qPCR DSGreen Standard	500 rxn	845-AS-1320500

4 Storage conditions

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 Delivered components

Component		Description
innuMIX qPCR DSGreen Standard		2x concenctrate (No ROX)

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

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

Reagent	Volume (1 rxn)
2x innuMIX qPCR DSGreen Standard	10 µl
Forward Primer	0.2 - 1 µM
Reverse Primer	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 and Detection*	50 - 68 °C	30 - 60 sec

* Detection in Green Channel: refer to instrument instructions

Melt Analysis: refer to instrument instructions

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

9 Hints and Notes

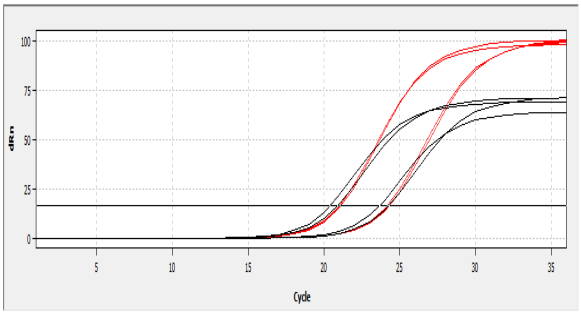
- For efficient amplification under fast cycling conditions we recommend amplicon lengths between 80 bp and 300 bp.
- The shorter the amplicon length the faster the reaction can be cycled.
- qPCR is a very sensitive DNA amplification reaction, therefore care should be taken to eliminate the possibility of contamination with any foreign DNA templates or PCR products.

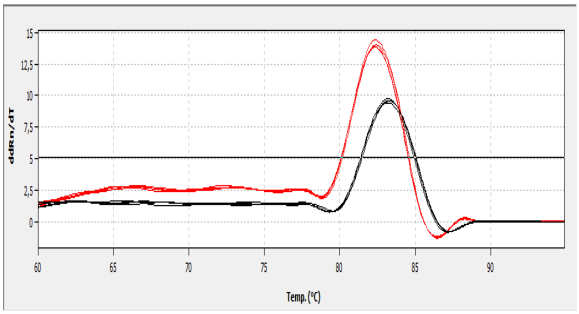
10 Application examples

Amplification of a “simple” sample (plasmid DNA) and a high fragmented and salt contaminated sample after a bisulfite conversion in comparison to a competitors SybrGreen MasterMix :

Amplification of Plasmid DNA

Amplification Plot





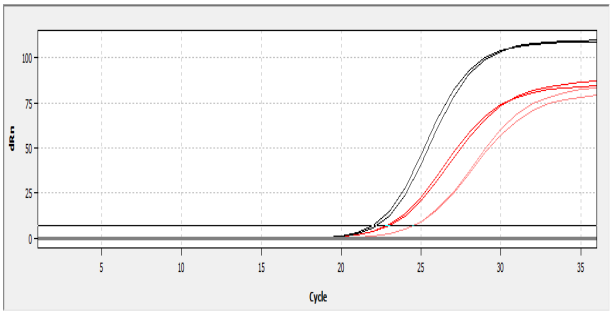
Melting Curve

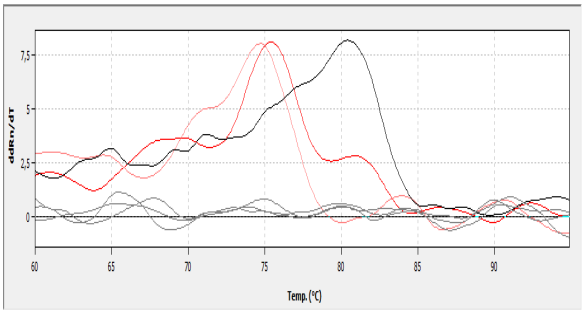
Red trace *= innuMIX qPCR DSGreen Standard*

Black trace *= Competitor Mix*

Amplification of bisulfite converted Blood DNA (CFF-Fragment)

Amplification Plot





Melting Curve

Red trace *= innuMIX qPCR DSGreen Standard, blood DNA before bisulfite conversion*
Light red trace*= innuMIX qPCR DSGreen Standard, blood DNA after bisulfite conversion*
Black trace *= Competitor Mix blood DNA before bisulfite conversion*
Light black trace*= Competitor Mix blood DNA after bisulfite conversion*

The results show, that innuMIX qPCR DSGreen Standard Mix unlike other MasterMixes does not lose the amplification ability by impure templates.

11 Related products

Product	Order Number
innuMIX qPCR SyGreen Sensitive	845-AS-1310100
innuMIX Green PCR MasterMix	845-AS-1400100
innuMIX qPCR MasterMix Probe	845-AS-1200100
innuMIX Standard PCR MasterMix	845-AS-1700100
innuDRY Standard PCR MasterMix	845-AS-2100100
innuDRY qPCR MasterMix Probe	845-AS-1900100

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