Instructions for Use
Life Science Kits & Assays

innuMIX Green PCR MasterMix

analytikjena
An Endress+Hauser Company

1 Product specifications

The 2x Master Mix contains two dyes (Bromophenol Blue and Orange G) to monitor migration progress during electrophoresis. The density of Master Mix is sufficient for direct loading onto agarose gels. The dyes absorb between 225 – 300 nm, making standard A260 readings to determine DNA concentration unreliable.

The Master Mix contains highly purified recombinant Taq DNA polymerase, highest quality dNTP's and optimal PCR reaction buffer components, thus only the template and PCR primers have to be add. The final reaction volume should be reached with PCR-grade water.

Taq DNA polymerase is thermal stable highly processive 5´ → 3´ DNA polymerase with low 5´ → 3´ exonuclease activity and lacks 3´ → 5´ exonuclease (proofreading) activity.

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.

0.1 ng of lambda DNA was amplified using specific primers to produce a distinct 4 kb band.

3 Product and order number

Name	Amount	Order-no.
innuMIX Green PCR MasterMix	100 rxn	845-AS-1400100
innuMIX Green PCR MasterMix	200 rxn	845-AS-1400200

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 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 in a thin-walled PCR tube, on ice.

Reagent	Volume (1 rxn)
PCR-grade H_2O	Variable (add to a final vol. of 20 µl)
2x innuMIX Green PCR MasterMix	10 µl
Forward Primer	0.2 - 1 µM
Reverse Primer	0.2 - 1 µM
Template DNA	1 - 100 ng/µl (≤ 1 µg)
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	25-40	Denaturation	95 °C	30 - 60 sec
		Annealing	50 - 68 °C	30 - 60 sec
		Elongation	72 °C	1 - 4 min
3	1	Final elongation	72 °C	5 - 10 min

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

8 Hints and Notes

- Keep the reaction tubes on ice as long as possible.
- Transfer samples from ice to the thermal cycler.

Note: If a thermal cycler without Sample Protection System is used, wait until denaturation temperature of about 94 °C has been reached.

Reaction conditions (incubation temperatures and times, concentrations of template DNA, primers, magnesium ions and enzyme) are depending on the used template and primers.

9 Related products

Product	Order Number
innuSTAR 1 kb DNA Ladder Express	845-ST-2010100
innuSTAR 100 bp DNA Ladder Express	845-ST-1010100

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