

# Instructions for Use

## Life Science Kits & Assays



**innuMIX Standard PCR MasterMix**

## 1 Product specifications

The innuMIX Standard PCR MasterMix contains all reagents required for routine high throughput PCR amplifications up to 5 kb sized DNA targets

The Master Mix contains an aptamere blocked hot start DNA polymerase, highest quality nucleotides (dATP, dCTP, dGTP, dTTP) and optimal PCR reaction buffer components, thus only the template and PCR primers have to be add. The concentration of MgCl<sub>2</sub> in the Master Mix is already suitable. The final reaction volume should be reached 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 Standard PCR MasterMix	100 rxn	845-AS-1700100
innuMIX Standard PCR MasterMix	200 rxn	845-AS-1700200

### 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 for 1 reaction

Reagent	Volume (1 rxn)
2x innuMIX Standard PCR MasterMix	10 $\mu$ l
Forward Primer	0.2 - 1 $\mu$ M
Reverse Primer	0.2 - 1 $\mu$ M
Template DNA	1 - 100 ng/ $\mu$ l (max. 1 $\mu$ g)
PCR-grade H <sub>2</sub> O	add to a final vol. of 20 $\mu$ l
Total volume	20 $\mu$ l

- 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.

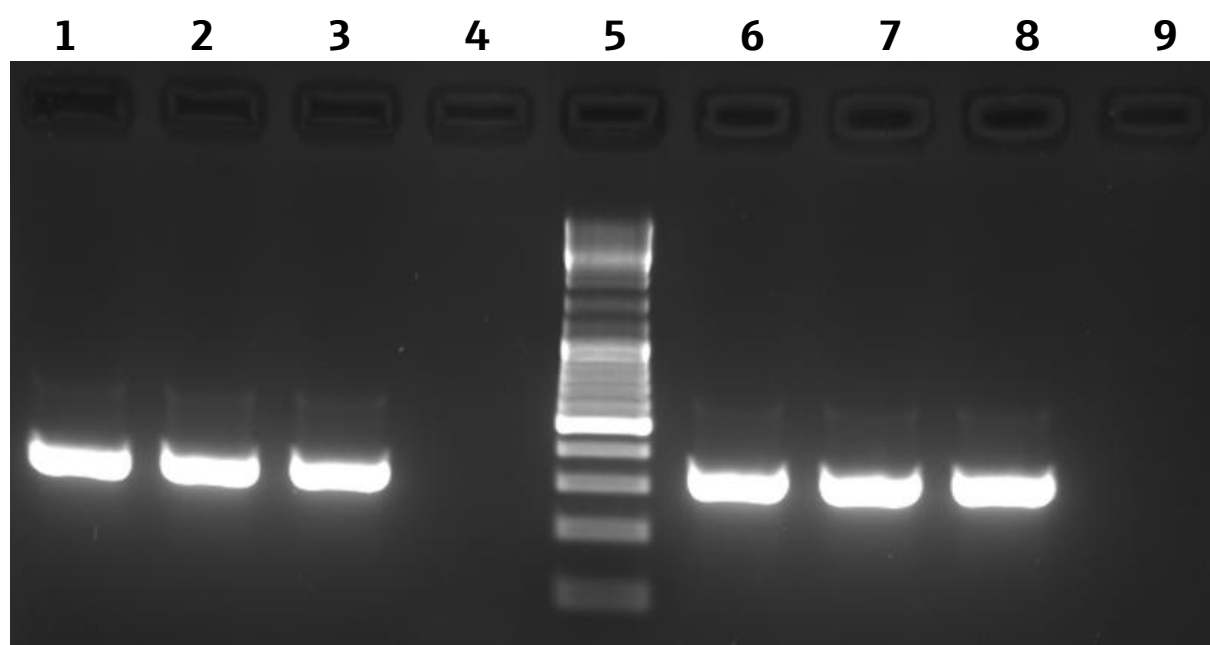
## 7 PCR conditions

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 Application examples

Detection of the amplified products of a DNA fragment using the innuMIX Standard PCR MasterMix and fresh mixed in-house PCR chemistry (innuTaq HOT-A DNA Polymerase, 50x inNucleotide Mix and appropriate Buffer with  $MgCl_2$ -Solution).



Lane	Description
1-3	DNA amplification using in-house PCR chemistry
4	NTC using in-house PCR chemistry
5	DNA ladder
6-8	DNA amplification using innuMIX Standard PCR MasterMix
9	NTC using innuMIX Standard PCR MasterMix

## 9 Related products

Product	Order Number
innuSTAR 100 bp DNA Ladder Express	845-ST-1010100
innuSTAR 1 kb DNA Ladder Express	845-ST-1020100
6x Loading Dye Bromophenol Blue	845-ST-3010006
6x Loading Dye Orange G	845-ST-4010006

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