



Uptima High-Quality Kits

For contamination
control

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For contamination control

Uptima offers novel and high-quality enzymes with unique combination of low temperature activity, as well as heat lability, to facilitate new possibilities in molecular biology applications.



**PCR Decontamination
Kit**



**Heat&Run gDNA
Removal Kit**

PCR Decontamination Kit

- ✓ Reduces background and improves target detection
- ✓ No negative effect on PCR sensitivity
- ✓ Efficient for ordinary PCR and probe-based qPCR mixes



“For effective removal of DNA contamination from PCR master mixes”

PCR is a sensitive method for detecting presence of DNA in both research and diagnostics. Taq polymerases are frequently contaminated with *E. coli* DNA, which might cause reduced sensitivity and false positives when small amounts of bacterial DNA are targeted. Other sources of contamination might be dNTPs, buffer components and primers/probes, as well as DNA introduced during handling.

Description

Uptima' PCR Decontamination Kit offers an easy and affordable solution for removal of contaminating DNA. It utilizes a double-stranded specific dsDNase to remove contaminating DNA. The specificity of dsDNase allows decontamination to occur with primers and probe present. In addition, it is efficient for some SYBR based qPCR mixes.

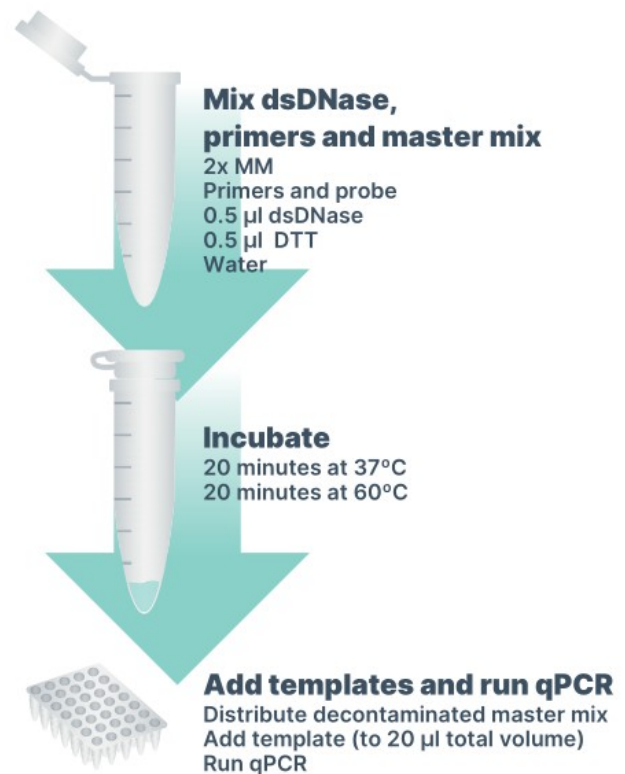
The dsDNase is irreversibly inactivated by heating to 60°C in presence of DTT, thus ensuring that any template added after inactivation remains safe from digestion.

Specifications

Source	dsDNase is recombinantly expressed in yeast
Contents	dsDNase (100/500 reactions) DTT (Inactivation aid)
Quality control	Tested for absence of RNase
Storage	Store at ≤ -20°C

Protocol

The PRC decontamination protocol is designed for removing contaminating DNA from 20 µl reactions but can be scaled to other convenient volumes.



Contaminating bacterial DNA is present in most 2x qPCR master mixes

Contaminating bacterial DNA in commercial qPCR master mixes might cause false positive results when using qPCR for detection or quantification of minor amounts of bacterial DNA. We used an *E. coli* 23S primer/probe set to detect the presence of bacterial DNA in probe-based qPCR 2x master mixes from several suppliers. As shown in figure 1, contaminating bacterial DNA was found in all master mixes tested.

Decontamination of master mixes without reduction of sensitivity

Contaminating DNA is mainly a problem in high-sensitivity applications, where low-abundant DNA are targeted. Because of this, any loss of sensitivity in the qPCR assay caused by the decontamination protocol is unacceptable.

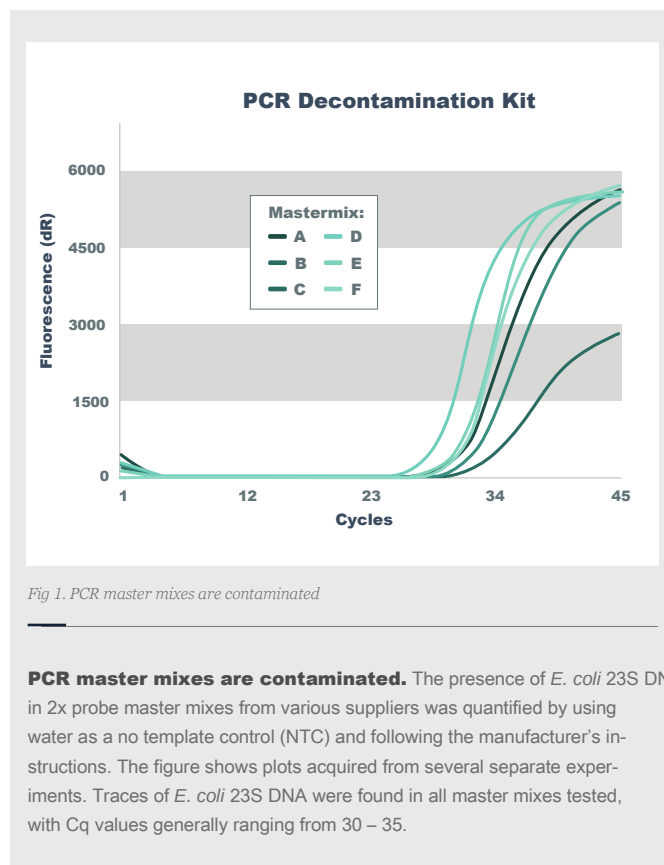


Fig 1. PCR master mixes are contaminated

PCR master mixes are contaminated. The presence of *E. coli* 23S DNA in 2x probe master mixes from various suppliers was quantified by using water as a no template control (NTC) and following the manufacturer's instructions. The figure shows plots acquired from several separate experiments. Traces of *E. coli* 23S DNA were found in all master mixes tested, with Cq values generally ranging from 30 – 35.

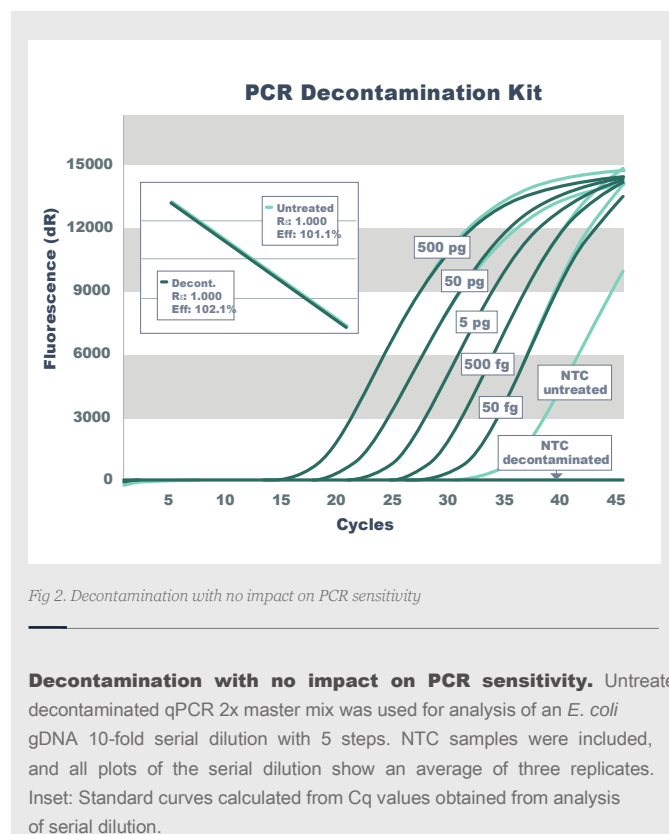


Fig 2. Decontamination with no impact on PCR sensitivity

Decontamination with no impact on PCR sensitivity. Untreated and decontaminated qPCR 2x master mix was used for analysis of an *E. coli* gDNA 10-fold serial dilution with 5 steps. NTC samples were included, and all plots of the serial dilution show an average of three replicates. Inset: Standard curves calculated from Cq values obtained from analysis of serial dilution.

In figure 2, the performance of decontaminated master mix was compared to untreated master mix.

A 5-step 10-fold serial dilution of *E. coli* gDNA was analysed using qPCR, which returned positive NTC. The PCR Decontamination Kit reduced the amount of contaminating DNA to levels below the detection limit of a 45-cycle qPCR experiment in the NTC samples. Treatment with the PCR Decontamination Kit did not significantly alter the Cq-levels obtained when analysing any of the dilutions of *E. coli* gDNA.

The Cq values presented was used to create standard curves (inset graph). No significant changes in either efficiency or R₂ between untreated and decontaminated master mix were observed.

Heat&Run gDNA Removal Kit

- ✓ Single-tube protocol
- ✓ Suitable for qPCR settings
- ✓ Increase cDNA yield



“For removal of gDNA contamination from RNA preparations”

Genomic DNA can be a challenge when purifying RNA. Many RNA purification methods leave traces of gDNA, which can cause problems in later analysis.

Description

The Heat&Run gDNA Removal Kit is designed for removal of contaminating gDNA from high-quality RNA prior to reverse transcription in qPCR.

The kit is based on the recombinant heat labile dsDNase (HL-dsDNase), which is irreversibly inactivated at low temperatures. After DNase treatment the HL-dsDNase is easily inactivated by a moderate rise in temperature (58°C). Genomic DNA removal and reverse transcription can be performed in the same tube, thereby minimizing pipetting steps and reducing hands-on time.

Specifications

Source	HL-dsDNase is an engineered version of dsDNase
Contents	HL-dsDNase (50/250 reactions) 10X Reaction buffer
Quality control	Tested for absence of RNase
Storage	Store at ≤ -20°C

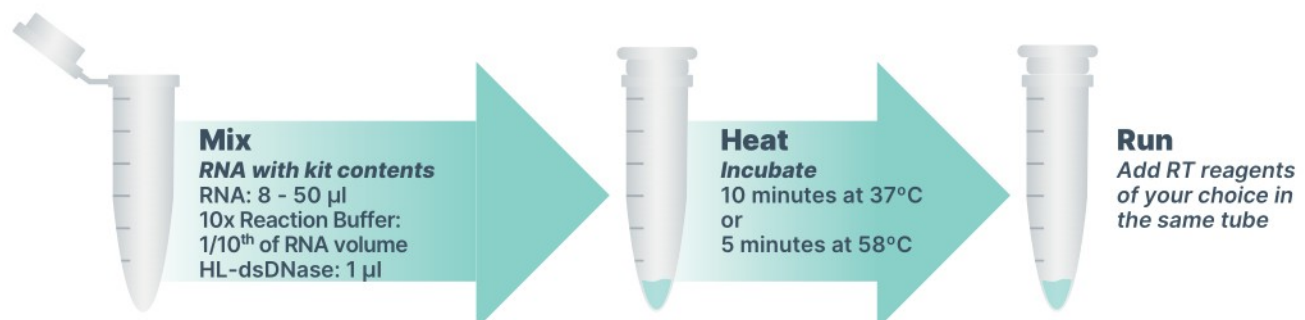
Protocol

Heat&Run gDNA Removal Kit can be used for RNA preps in samples with high-quality RNA, see figure below.

Purification of nucleic acids is required prior nuclease treatment.

Mix your RNA with Heat&Run, incubate for 10 min at 37°C, and then inactivate the dsDNase for 5 min at 58°C.

Run your RT!



Ordering information

	Article No.	Pack size
PCR Decontamination Kit	1D8980	100 rxn
	1D8981	500 rxn
Heat&Rur® gDNA Removal Kit	1D8970	50 rxn
	1D8971	250 rxn

Your Partner to deliver novel solutions for genomics and proteomics

Quality

Uptima is dedicated to the quality of our products

Additional information

We are pleased to provide support using our protocols or customized protocols. For more information, please check our website www.interchim.com or contact us.