

PCR Decontamination Kit

Catalog #ENZ-KIT137

- Conveniently remove DNA contaminations from PCR mastermixes
- Reduce background: improve target detection
- No negative effect on PCR sensitivity
- Efficient for ordinary PCR and probe based qPCR mixes

Product Description

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 dNTP's, buffer components and primers/probes, as well as DNA introduced during handling.

The PCR Decontamination Kit from Enzo Life Sciences offers an easy and affordable solution for removal of contaminating DNA. It utilizes a dsDNase to remove contaminating DNA. The double-strand specific property allows decontamination to occur with primers and probe present. Also, dsDNase is irreversibly inactivated by heating to 60°C in presence of DTT, ensuring that any template added after inactivation remains safe from digestion.

Kit Contents:

- dsDNase (100/500 reactions)
- DTT (Inactivation Aid)

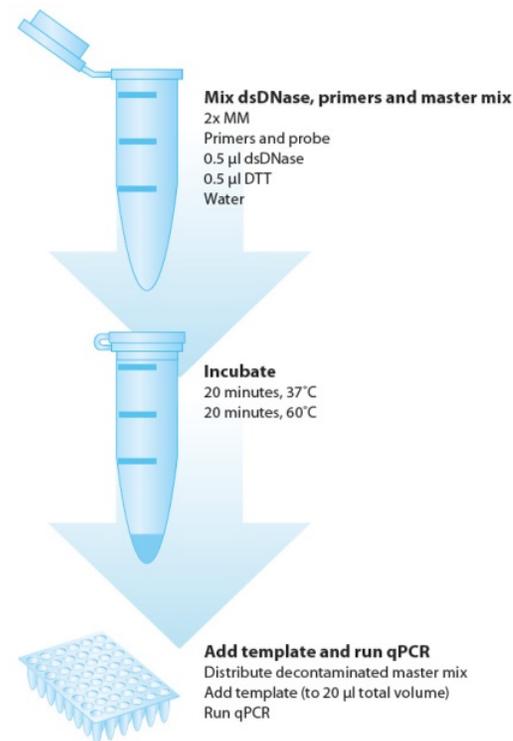
Storage Conditions: Store at -20°C.

Sample Material: The kit can be used to reduce contaminating DNA in both ordinary PCR and probe based qPCR mixes. Also efficient for some SYBR based qPCR mixes.

Quality Control: The kit is tested for absence of RNase.

Decontamination Protocol

The decontamination protocol is designed for removing contaminating DNA from 20µl reactions, but can be scaled up or down by adjusting the volume of the kit contents.



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.

The performance of decontaminated master mix was compared to untreated master mix.

We used water (NTC) and a 5-step 10-fold serial dilution of gDNA as template. 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 (**Figure 2**). Treatment with the PCR Decontamination Kit did not significantly alter the C_q-levels obtained when analyzing any of the dilutions of *E. coli* gDNA (**Figure 2**).

The C_q values presented in **Figure 2** was used to create standard curves (inset, **Figure 2**). No significant changes in either efficiency or R_q between untreated and decontaminated master mix were observed. Reducing 23S decontamination to levels below the detection limit without affecting PCR sensitivity was also confirmed in all master mixes presented in **Figure 1**.

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PCR master mixes are contaminated

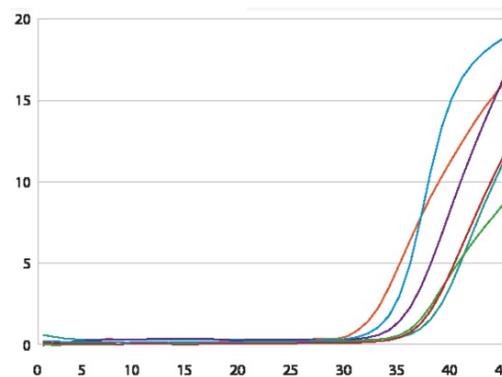


Figure 1: The presence of *E. coli* 23S DNA in 2x probe master mixes from various suppliers was quantified by using water as template (NTC) and following the manufacturer's instructions. The figure shows plots acquired from several separate experiments. Traces of *E. coli* 23S DNA were found in all master mixes tested, with C_q values generally ranging from 30 – 35.

No effect on PCR sensitivity

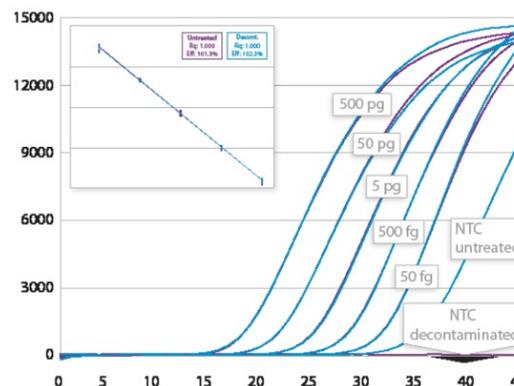


Figure 2: Untreated and decontaminated qPCR 2x master mix was used for analysis of an *E. coli* gDNA 10-fold serial dilution with 5 steps. NTC samples were included, and all plots of the serial dilution show an average of three replicates. Inset: Standard curves calculated from C_q values obtained from analysis of serial dilution.