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Additional Notes
The decontamination protocol can also be performed in individual tubes by completing step 3 of the protocol prior to step 2.

The dsDNase is highly specific for double stranded DNA, thereby avoiding digestion of primers and probe. However, if primers/probe forms double stranded sections, either internal or with each other, digestion of primers/probe might occur. Performing decontamination in low volumes (i.e. adding template in 5 µl) reduces the risk of probe degradation. If the problem persists, add primers/probe after inactivation of dsDNase.

There are relatively large differences in buffer compositions among various master mixes. Consequently some discrepancies in efficiency of dsDNase depending on the master mix used must be expected.

Avoid using more than 0.5 µl dsDNase per 20 µl qPCR reaction, as higher volumes might cause inhibition of the PCR reaction.

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PCR Decontamination Kit

This document refers to the following product numbers (P/N) supplied by ArcticZymes:
80400-000
80400-100

Properties
The PCR Decontamination Kit from ArcticZymes is designed for removal of contaminating DNA from commercial PCR master mixes prior to addition of template. The set utilizes ArcticZymes double-strand specific DNase (dsDNase), which is easily inactivated at 60°C in presence of DTT. This is below the temperatures necessary for activation of hot-start Taq polymerases. The double-strand specific properties of dsDNase allow treatment of master mixes with primers and probe present.

Source
dsDNase is recombinantly expressed in yeast.

Kit Contents
dsDNase (1 vial) and DTT (1 vial) sufficient for 100 reactions (20 µl final qPCR reactions).

Protocol: 2x qPCR master mix

The protocol describes decontamination of qPCR reactions with a volume of 20 µl including template. For other reaction volumes, adjust amounts of kit contents correspondingly.

Set up the decontamination reaction as follows:

1. Combine all your PCR components except template (2x master mix, primers/probe and water) in a tube large enough to pool all your qPCR reactions. Add 0.5 µl dsDNase and 0.5 µl DTT per qPCR reaction.

Note: The template volume should ideally range from 2–5 µl per reaction. Use 0.5 µl of each dsDNase and DTT regardless of intended template volume.

2. Mix well, spin down and decontaminate at 37°C for 20 minutes followed by dsDNase inactivation at 60°C for 20 minutes.

Note: If a heating block without lid is used, spin down content prior to increasing the temperature to 60°C.

3. Distribute decontaminated master mix to PCR wells/tubes.

4. Add template to a total volume of 20 µl.

5. Run qPCR.

Quality Control
dsDNase is tested for absence of RNase.

Sample Material

The PCR Decontamination Kit is designed for use in commercial 2x qPCR master mixes. The kit is proven to reduce the amount of decontaminating DNA from master mixes with both Probe and SYBR based detection. Differences in buffer compositions in master mixes might result in variable efficiency of dsDNase. The kit has in general higher efficiency in probe mixes compared to SYBR mixes.

For end-point PCR or other concentrations of master mixes, adjustments of protocol may be necessary. Example: if the master mix is more concentrated than 2x, adjust to 2x before decontaminating.

Add template and run qPCR

Distribute decontaminated master mix
Add template
Run qPCR

Mix dsDNase, primers
and master mix

2x master mix
Primers and probe
dsDNase
DTT
Water

Incubate
20 minutes, 37°C
20 minutes, 60°C

Note:

Expiration
See label.

Storage
Store at -20°C. Aliquotation of DTT is recommended to avoid repeated freeze-thaw cycles.

Example: if the master mix is more concentrated than 2x, adjust to 2x before decontaminating.

Note: The template volume should ideally range from 2–5 µl per reaction. Use 0.5 µl of each dsDNase and DTT regardless of intended template volume.

Note: If a heating block without lid is used, spin down content prior to increasing the temperature to 60°C.

3. Distribute decontaminated master mix to PCR wells/tubes.

4. Add template to a total volume of 20 µl.

5. Run qPCR.