Preparation of DNA-free RNA prior to RT-PCR

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INTRODUCTION

The fluorescent DNA-binding dye used in qRT-PCR binds to all kinds of double stranded DNA. Therefore, to prevent false-positive results, the RNA is treated with DNase to remove remaining DNA.

MATERIALS

RNA extracted from *E. coli* MG1655 (wild-type) and MG1655 (*kdpA4*)

REAGENTS

- RNase-free deoxyribonuclease I (Fermentas, St.Leon-Rot)
  includes: DNase I, 10x reaction buffer with $MgCl_2$ and EDTA 25 mM
- DEPC (Diethyl pyrocarbonate) (Roth, Karlsruhe)

REAGENTS SET-UP

- **DEPC-H_2O:** Add 1 ml DEPC to 1 l dH_2O, stir over night under the hood and autoclave

EQUIPMENT

- Reaction tubes 1.5 ml (RNase-free)
- Thermomixer (Eppendorf)
- NanoDrop Spectrophotometer (Peqlab)

PROCEDURE

TIMING ~ 1h. Depending on the number of samples

NOTE: work on ice with RNase-free, sterile material!

The RNA was digested with RNase-free deoxyribonuclease I following the instructions of the users manual for DNA-free RNA for RT-PCR

Briefly:

- Incubate 5 µg RNA together with 5 µl 10x reaction buffer with $MgCl_2$, 5 µl DNase I and DEPC-treated water (to 45 µl) at 37℃ for 30-45 min
- Add 5 µl 25 mM EDTA and incubate at 65℃ for 10 min
- Measure the RNA concentration at 260 nm with NanoDrop Spectrophotometer
- Use the prepared RNA as template for reverse transcriptase

REFERENCES

Instruction manual: Deoxyribonuclease I (DNase I), RNase-free (Fermentas)