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DI-Check Free DNA Degradation kit

MBK0080-FDD _ 250 extractions

INTRODUCTION AND PRODUCT DESCRIPTION

The DNApure Water Isolation Kit (MBK0080-Diatheva) can be combined with Di-Check Free DNA Degradation kit for the elimination of free DNA.
The degradation of free DNA is performed by a specific enzyme and its activation buffer. This enzymatic treatment ensures that samples are devoid of free DNA prior to DNA extraction, allowing the selection, purification and recovery of genomic DNA deriving from intact and living cells. The enzyme is inactivated by thermal lysis step and Lysis Buffer use.

CONTENTS AND STORAGE

Component	Unit and Volume
Activation Buffer	1 x 125 ml
Free DNA Degradation Reagent	1 x 0.5 ml

Store the Activation Buffer at room temperature and the Free DNA Degradation Reagent at -20°C.
If properly stored, see the expiration date for the stability of the component.

ADDITIONAL EQUIPMENT AND MATERIAL REQUIRED

Water filtration:

- Filtration system with disposable sterile funnels,
- Polycarbonate filters with a nominal porosity of 0.45 µm. Do not use a membrane containing cellulose,
- Sterile metallic inox tweezers,
- Class II safety cabinet,
- Powder free gloves.

DNA extraction:

- Water bath at 37 °C ± 2°C
- Water bath at 95°C ± 5°C, preferably with a cap,
- Vortex,
- Centrifuge for 1.5-2 ml tube for centrifugation at 500 x g and 1000 x g,
- Magnetic stir plate,
- Micropipettes and filter tips,
- Powder free gloves.

PRECAUTIONS

- The test must be performed by specialised, trained and authorised staff,
- Do not use reagents after the expiry date printed on the label,
- Use gloves (powder free) during the whole procedure,
- Change gloves often, especially if you suspect a possible contamination of them,
- It is suggested to perform a negative control of the method by following the complete procedure on a sample volume from 200 mL to 1 L of *Legionella*-DNA free sterile solution,
- Filter funnels must be sterilized in an autoclave and if made metal, flamed prior to use,
- After DNA extraction clean working space periodically with at least 5% sodium hypochlorite or another decontaminant agent,
- It is suggested to provide separate and dedicated spaces, material and equipment for pre- and post-PCR amplification stages.

PROCEDURE

1. SAMPLING

Please refer to the instruction for use of DNApure Water Isolation Kit (MBK0080-Diatheva):

Water samples shall be collected in sterile containers with all necessary precautions. Please perform sampling according to general standards for Legionella detection and quantification. Preferably start the analysis immediately after the sampling. If water samples are treated with oxidizing biocide or other wait at least 48 hours before collecting water samples and use sterile containers with an appropriate inactivating agent.

2. WATER FILTRATION

Please refer to the instruction for use of DNApure Water Isolation Kit (MBK0080-Diatheva):

1. Rinse the filtration ramp with 100 ml of Legionella DNA-free water and decontaminate the ramp by burning with alcohol. Make sure the ramp is dry and not hot before positioning the filter. This operation should be repeated after each filtered sample.
2. Carefully remove one membrane filter from the packaging. Insert the membrane on the filtration apparatus and filtrate the amount of water sample.
Record the volume of sample filtered this is required to calculate the results.

3. DEGRADATION OF FREE DNA AND DNA EXTRACTION

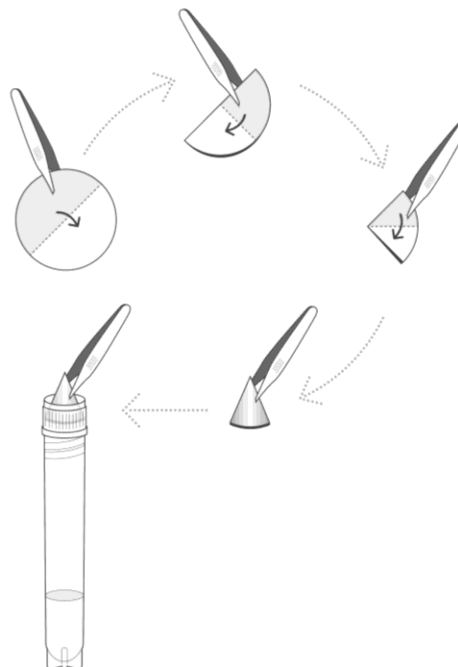
Before starting:

- Pre-heat water bath at 37 ± 2°C for the Free DNA degradation step
- Pre-heat water bath at 95 ± 5 °C for the DNA extraction step
- Prepare the number of Cryotube^{TM} vials corresponding to the number of samples by pipetting **0.5 ml of Activation Buffer** and **2 µl of the Free DNA Degradation Reagent** in each Cryotube^{TM} (**Free DNA Degradation Buffer**)
- Insert the number of DNApure Columns required in the 1.5 ml Tubes (provided)
- All centrifugation steps must be carried out at room temperature

1. Using the sterile tweezers fold the filter in two, three times in order to obtain a cone, as shown in Figure 1

2. Transfer the membrane into a Cryotube^{TM} vial containing the Free DNA Degradation Buffer
Note: the membrane filter must be insert in the vial with the point of the cone oriented to the top of Cryotube^{TM} vial (Figure 1)

Figure 1



3. Verify that the filter is soaked in the Free DNA Degradation Buffer

4. Flick the tube gently with your finger at least 10 times for homogenization (do not vortex)
Note: verify that the filter is soaked in Free DNA Degradation Buffer prior to proceed with step 5

5. Incubate the samples in water bath at 37 ± 2°C for 30 minutes

6. Add **1.5 mL Lysis Buffer** of the DNApure Water Isolation kit (MBK0080 - Diatheva) to inactivate Free DNA Degradation Buffer and for DNA extraction
Note: mix thoroughly the Lysis Buffer while pipetting the buffer into CryotubeTM vials on a magnetic stir plate (medium speed) in order to keep it in suspension and collect the resin

7. Vortex for 20 seconds at maximum speed
Note: verify that the filter is completely soaked in Lysis Buffer prior to proceed with step 8

8. Incubate the samples in water bath at 95 ± 5°C for 15 minutes to complete the inactivation of the Free DNA Degradation Buffer and for DNA extraction

9. Continue to step 5 of "PROCEDURE", "3. DNA EXTRACTION" of the Instruction for use of the DNApure Water Isolation kit (MBK0080 - Diatheva)

4. DETERMINATION OF CONVERSION FACTOR (F)

To calculate the amount of target bacterium in the water sample analyzed using the DI-Check Free DNA Degradation Kit, it is necessary to consider (1) the volume of sample filtered and (2) the **F** conversion factor.
The **F conversion factor** of the DNApure Water Isolation kit when using the DI-Check Free DNA Degradation Kit and when 5 µl of DNA extract is analyzed in qPCR, is **68**. This value is a conversion factor between No. of genome units per well to No. of genome units per litre and considers all the dilution steps applied during the extraction process:

- 200 µl of the lysate is purified out of the 1700 µl of the supernatant→ 1700/200 = 8.5
- The final volume of DNA extract is 40 µl, if 5 µl is analyzed in qPCR → 40/5=8

F conversion factor → 8.5 x 8 = **68**

The No. of genome units (GU) of the target DNA (PCR results) should be multiplied by 68 and divided by the volume of water sample filtered (expressed in litre), as indicated in the formula below:

$$\frac{\text{n° of genome unit x } \mathbf{68}}{\text{sample volume (L)}}$$

Example: a water sample of 500 ml is analyzed for *Legionella* spp. enumeration using the DI-Check Free DNA Degradation kit, then 5 µl of the purified DNA is used for the real-time PCR reaction. The PCR result is 30 GU/well.

$$\frac{\text{30 GU/well x 68}}{\text{0.5 L}} = \text{4080 GU of } \textit{Legionella} \text{ per litre of water sample}$$