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# DNApure Water Isolation kit

# MBK0080 - 100 extractions

## INTRODUCTION AND PRODUCT DESCRIPTION

The DNApure Water Isolation kit provides a convenient and efficient procedure for DNA extraction of gram- bacteria present in water samples. The kit can isolate genomic DNA from a wide variety of filtered water samples, such as drinking water, sanitary hot water, cooling tower water, swimming pools and different clear water samples not containing overmuch suspended particles. Lysis occurs by thermal shock at 95±5°C and vortex mixing in the Lysis Buffer, specifically formulated to remove contaminants that could be found in water samples. The presence of a resin which acts via ion exchange limits destruction of the DNA by inactivating nucleases and chelating heavy metals that may damage DNA.
The lysate is than purified and recovered using the DNApure Column that provides excellent DNA quality and yield. The purified DNA is suitable for a real-time PCR detection and quantification.
The kit was specifically developed and tested for the DNA extraction of *Legionella* bacteria from water samples, in compliance with ISO/TS 12869:2019 requirements.

## CONTENTS AND STORAGE

| Component | Volume or unit |
|---|---|
| Lysis Buffer | 2 x 100 mL |
| Elution Buffer | 1 x 3.5 mL |
| DNApure Column | 1 x 100 units |
| 1.5 mL Tubes | 2 x 100 units |
| Cryotube™ vials | 2 x 50 units |

Store all kit components at +2 to 8°C except for DNApure Column that must be stored at room temperature. If properly stored, see the expiration date for the stability of the kit.

## 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 95°C ± 5°C, preferably with a lid,
- Vortexer,
- 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 of metal, flamed prior to use,
- After DNA extraction clean working space periodically with at least 5% sodium hypochlorite or other decontaminant agent,
- It is suggested to provide separate and dedicated spaces, material and equipment for pre- and post-PCR amplification stages.

# PROCEDURE

## 1. SAMPLING

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

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 filtered sample for later calculation of results.

## 3. DNA EXTRACTION

Before starting:
- Pre-heat water bath at 95 ± 5°C for the DNA extraction step.
- Prepare the number of Cryotube™ vials corresponding to the number of samples by pipetting 2 mL of Lysis Buffer in each Cryotube™ vial using a 1000 µL micropipette.
*Note: mix thoroughly the Lysis Buffer while pipetting the buffer into Cryotube™ vials on a magnetic stir plate (medium speed) in order to keep it in suspension and collect the resin.*
- Insert the required 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 two to three times in order to obtain a cone, as shown in Figure 1.
2.Transfer the membrane into a Cryotube™ vial containing 2 mL of Lysis Buffer.
*Note: the membrane filter must be inserted in the vial with the point of the cone oriented to the top of Cryotube™ vial (Figure 1).*

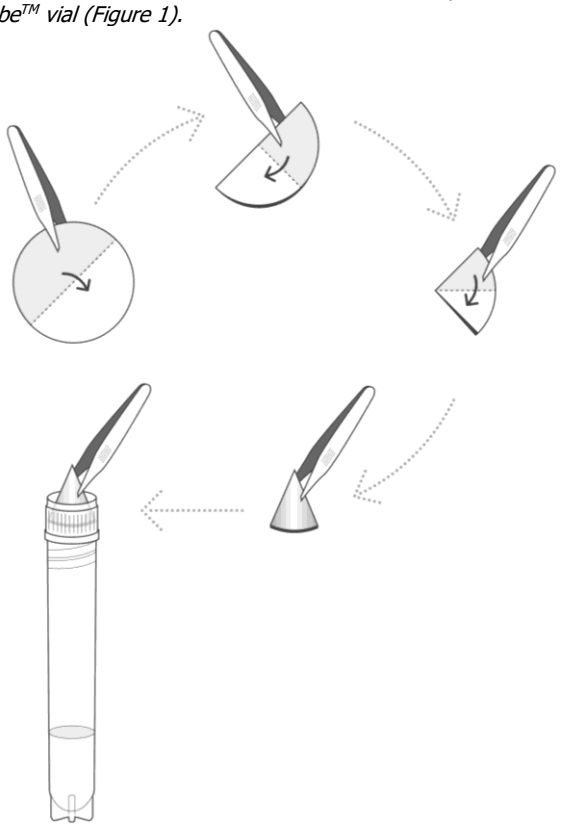

Figure 1

3.Vortex for 20 seconds at maximum speed.

*Note: verify that the filter is completely soaked in Lysis Buffer before proceeding to step 4.*

4. Incubate the samples in water bath at 95 ± 5°C for 15 minutes.
5. Vortex for 20 seconds at maximum speed.
6. Using a 1000 µL filter tip press the filter into the wall of Cryotube™ vial to recover all the lysate. Discard the filter.
7. Incubate at room temperature for 20 minutes in order to cool down the lysate and to pellet the resin at the bottom of Cryotube™ vial. During the incubation avoid to move the tube. Alternatively, is it possible to centrifuge the Cryotube™ at 900 x g for 3 min in a centrifuge adapted for 4.5 mL tube. Verify that the tubes are at room temperature before starting the purification step.
8. Pipette 200 µL of the supernatant into the DNApure Column (insert in the provided 1.5 mL tube) taking care not to touch the membrane with the pipette tip. Seal with attached cap.

*Note: Do not pipette the pellet formed by the resin. If you add the resin, release the sample in the Cryotube™ vial and wait again (or centrifuge at 900 x g for 3 min) until the resin is decanted.*

9. Place the DNApure Column into the centrifuge and align the cap strap toward the centre of the rotor as indicate in the Figure 2. Centrifuge for 20 minutes at 500 x g.

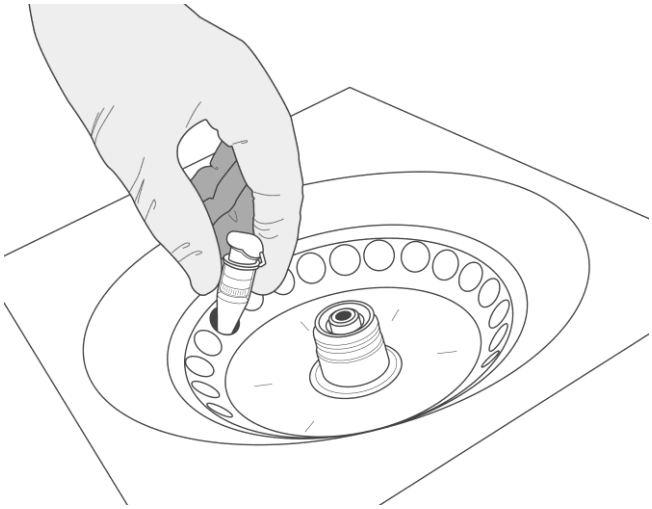

Figure 2

*Note:*
- *Counterbalance the centrifuge with a similar device and verify that all the DNApure Columns are correctly placed in the rotor. Check that all the supernatant is filtered though the column. If not, perform another 3 minutes centrifuge.*
- *If clogging occurs in the DNApure Column, increase the centrifugation time without changing the centrifugation speed.*

10. Add 30 µL of Elution Buffer in the centre of the membrane taking care not to touch the membrane with the pipette tip.
11. Discard the 1.5 mL Tube and place a new 1.5 mL Tube (provided) over the top of the DNApure Column. Turn the assembled device upside down (Figure 3) and place in centrifuge, aligning open cap towards the centre of the rotor, counterbalance with a similar device and centrifuge for 3 minutes at 1000 x g.

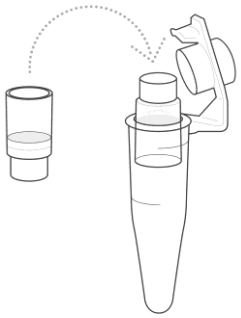

Figure 3

12. Throw away the DNApure Column. The eluate contains pure genomic DNA.
DNA extracts shall be stored at 5 ± 3°C and analysed within 24 h of preparation. For long terms storage at -20°C is recommended.

## 3. DETERMINATION OF CONVERSION FACTOR (F)

To calculate the quantity of target bacteria in the water sample analyzed, it is necessary to take into account (1) the volume of the filtered water sample and (2) the conversion factor **F**.
The **F conversion factor** of the DNApure Water Isolation kit when 5 µL of DNA extract is analyzed in qPCR, is **64**. 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 1600 µL of the supernatant→ 1600/200 = 8
- The final volume of DNA extract is 40 µL, if 5 µL is analyzed in qPCR → 40/5=8

***F conversion factor*** → 8 x 8 = **64**

The No. of genome units (GU) of the target DNA (PCR results) should be multiplied by 64 and divided by the volume of water sample filtered (expressed in litre), as indicated in the formula below:

$$\frac{\text{n° of genome unit x } \mathbf{64}}{\text{sample volume (L)}}$$

Example: a water sample of 500 mL is analyzed for *Legionella* spp. enumeration, then 5 µL of the purified DNA is used for the real-time PCR reaction. The PCR result is 30 GU/well.

$$\frac{30 \text{ GU/well x } 64}{0.5 \text{ L}} = 3840 \text{ GU of } \textit{Legionella} \text{ per litre of water sample}$$

## 4. VALIDATIONS

DI-Check Legionella pneumophila
Certificate reference No. DTV 41/01-12/19, with end of validity 04-Dec-2027
WATER ANALYSIS METHODS
The method is validated for the detection and quantification of L. pneumophila in all types of water samples, by comparison to the reference method NF T90-471 (2015) and ISO/TS 12869 (2019) and according AFNOR certification's validation protocol for the detection and enumeration of L. pneumophila by PCR.
https://nf-validation.afnor.org/

Note to the purchaser:
Cryotube™ is a trademark of Nunc