DNA PURE-FLASH

DETAILED OPERATING MODE

GL BIOCONTROL – Company specialized in bioenvironmental risk management of water
9, avenue de l'Europe - CAP ALPHA - 34 830 CLAPIERS - FRANCE / Phone: +33.(0)9.67.39.35.20 - Fax: +33.(0)9.55.25.40.31
Email: contact@gl-biocontrol.com - Web: www.gl-biocontrol.com

1. INTRODUCTION

Water provides an ideal environment for the development of bacteria. Although most bacteria are not harmful to our health, some such as *Legionella pneumophila, Salmonella* or certain strains of *Escherichia coli* present public health risks. According to the WHO guidelines, it is strongly recommended to carry out regular checks on the microbiological quality of sanitary and industrial water (cooling towers...) in order to minimize the risks. This surveillance directly involves pathogenic bacteria or indicators of fecal contamination such as *Escherichia coli*.
Real-time PCR is an extremely powerful and fast tool used for the specific detection of bacteria in water. This methodology, now largely used, is already the subject of an NF standard and an ISO standard for the research of *Legionella* in sanitary and industrial water. To achieve optimum performances, the preparation of the sample and the extraction of DNA must be performed with precision as it is the most important step. Indeed, this step is essential for the effective elimination of PCR inhibitors without loss of extracted DNA.

The **DNA PURE-FLASH** kit provides a robust and reliable solution for the optimal extraction of DNA from bacteria while ensuring the removal of inhibitors for real-time PCR analysis.

2. KIT CONTENT

REAGENTS	QUANTITY PER KIT
Microtube of Lysis buffer, S1	96 x 1,3 ml
Dropper-bottle of Binding buffer, S2	4 x 2,0 ml
Tube of Elution buffer, S3	4 x 2,5 ml
Microtube of WCX resin tubes	96
Microtubes 2.0 ml	96
Microtubes 1.5 ml	96
Tapered tips for micropipettes	96

3. STORAGE OF REAGENTS

The *lysis buffer* (S1), the *binding buffer* (S2) and the *elution buffer* (S3) should be stored in the fridge, between 2°C and 8°C. In these conditions, each reagent can be used up to the expiration date.

4. EQUIPMENT AND MATERIAL REQUIRED (NOT PROVIDED IN THE KIT)

- Filtering manifold (ramp),
- Sterile funnels,
- Polycarbonate membranes 0.45 µm,
- Gloves,
- Micropipettes 100 µl and 1000 µl,
- Sterile tips for 100 µl and 1000 µl micropipettes,
- Thermomixer (temperature range: 25 ° C - 100 ° C / Orbital stirring ≥ 1500 rpm),
- Benchtop micro centrifuge.

Optional:

- Vacuum pump
- Ultrafiltration column

5. PRECAUTIONS

This test must be performed by personnel appropriately trained.

It is essential to use at least one negative control for each extraction series, preferably at the end of the series.

Do not use reagents past their expiration date.

6. PROTOCOL FOR THE TREATMENT OF WATER SAMPLES

a. Preliminary operations

1. Preheat the thermomixer or a heated bath to 95 ° C ± 5 ° C,
2. Prepare the number of *lysis buffer* tubes (S1) corresponding to the number of samples to be treated,
3. On a rack, prepare the number of WCX resin tubes corresponding to the number of samples to be treated.

b. Filtration of water samples

1. Decontaminate the filtering manifold with alcohol. Before handling, the ramp must be dry and cool. This step must be performed between each sample,
2. Place the membrane on the ramp and add a sterile funnel,
3. Filter the sample.

c. DNA extraction and purification

1. Gently fold the membrane in eight using sterile tweezers to obtain a cone,
2. Using a micropipette tip and tweezers, place the membrane into the Lysis buffer tube (S1) with the tip of the cone pointing upwards, towards the top of the tube,

   Note: the membrane must be completely submerged in the solution S1.

3. Incubate at 95 ° C ± 5 ° C for 15 minutes, stirring 1800 RPM,

   Note: this step can also be performed in a heated bath. Once the incubation is complete, prepare the thermomixer at 75 ° C ± 5 ° C for step n°13.

4. Gently remove the membrane, pressing it on the side of the tube and then discard the membrane,
5. Centrifuge the tubes 2 minutes in the microcentrifuge or allow the resin to decant for 5 minutes at room temperature,
6. Pipet 1.0 ml of supernatant containing the extracted DNA into a new 2.0 ml microtube,

   Note 1: if a fraction of the resin from the lysis solution (S1) is removed, re-deposit the supernatant in the tube and centrifuge again.

   Note 2: if the sample to be analyzed is too dirty (loaded with suspended matter) take less than 1.0 ml (ex: 800 µl). Take account of this volume in the Z factor for the final calculation (see chapter 7).

7. Add 2 drops of binding buffer (S2) and manually shake the tube by means of three repeated inversions,

   Note: in case one or two additional drops are added by mistake, the performance of the protocol will not be impacted.

8. With a micropipette, transfer the entire volume of solution into the WCX resin tube,
9. Stir the tube 20-30 seconds at 2 500 RPM with a vortex mixer,
10. Centrifuge for 30 seconds between 1 500 and 5 000 x g or allow the resin to decant for 2 minutes at room temperature,
11. Using a tapered "Gel-loading" tip placed on the syringe, remove all liquid. Start by removing the liquid above the resin and then plunge the tip inside the resin. Discard the liquid,

    Note: the tip must be changed between each sample. A vacuum pump can also be used.

12. With a micropipette, add 100 µl of elution buffer (S3) in the tube,

13. Mix using the thermomixer at 1 800 RPM, 75°C ± 5°C for 10 minutes,

14. Centrifuge between 1 500 and 5 000 x g for 1 minute,

15. Extract between 50 µl and 100 µl of the supernatant, taking care not to pipet resin. Transfer all the extract in a 1.5 ml tube supplied. The purified sample is stable for several months at -20 ° C.

Note: To avoid sampling resin, a gel-load tip may be used. If a fraction of resin is removed, re-deposit the supernatant in the tube and centrifuge again.

7. CALCULATION AND EXPRESSION OF RESULTS

a. The Z Factor for the DNA PURE-FLASH protocol

The Z factor transforms the GU (Genome Unit) in the well into GU in the filtered volume:

GU per well x Z Factor = GU in the volume filtered

The Z factor is specific of each extraction method because it depends on the fraction of sample purified (Z_1) at the extraction step, and on the fraction of sample analyzed (Z_2) by PCR. It is calculated as follows:

- **Extraction and purification step**: S1 solution is composed of 1.1 ml of liquid and 0.2 ml of Chelex. Only 1.0 ml of S1 supernatant is purified out of a total of 1.1 ml of S1.
  Thus, the fraction purified is 1/1.1 of the sample. In our equation, Z_1 equals 1.1.

- **PCR analysis step**: after the elution step (step 13), the microtube contains 200 µl of DNA extract. Only 5 µl are analyzed by PCR.
  Thus, the fraction analyzed is 5/200 or 1/40 of the sample. In our equation, Z_2 equals 40.

Therefore, the Z factor for the **DNA PURE-FLASH** extraction method is: $Z = Z_1 \times Z_2 = 1.1 \times 40 = 44$.

b. Limit of detection (LOD) and quantification (LOQ):

The LOD of the overall method (extraction / purification and detection) is calculated as follows:

$$\frac{\text{LOD qPCR} \times \text{Z} \times \text{D}}{\text{Filtered volume (L)}}$$

D represents the dilution factor if the DNA extract was diluted prior to PCR.

Same method for the LOQ of the global method:

$$\frac{\text{LOQ qPCR} \times \text{Z} \times \text{D}}{\text{Filtered volume (L)}}$$

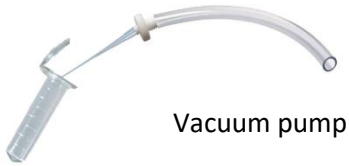
8. ALTERNATIVE PROTOCOLS

For each alternative protocol, it is imperative to take into account the changes in the calculation of the Z factor.

Conventional protocol with the elimination of the supernatant (step 11) by means of a vacuum pump for all matrices

Steps 1 to 10 and 12 to 15 are strictly identical to the conventional protocol. Only step 11 is different:

11. Remove the supernatant with a vacuum pump connected to a tapered tip.
Note: The tip must be changed between each sample.


Vacuum pump

The remainder of the protocol can be carried out according to the conventional protocol.

Calculation of the Z factor
The Z factor is strictly identical to the Z factor of the conventional protocol: $Z = Z_1 \times Z_2 = 1.1 \times 40 = 44$.

Protocol using an ultrafiltration column for clear water

Steps 1 to 14 are strictly identical to the conventional protocol. The remainder of the protocol can be carried out as follows:

15. Deposit 100 µl of the eluate located above the resin into the ultrafiltration column,
Note: To avoid drawing resin, a gel-loaded tip may be used. In the case where resin is removed, the supernatant is re-deposited in the tube and centrifuged again.

16. Centrifuge for 10 minutes at 6000 x g,

17. Put the column in a new microtube and discard the collector tube

18. Add 30 µl of elution buffer (S3) to the column.

19. Centrifuge for 3 minutes at 1000 x g.
Note: at this stage the tubes cannot be closed.

20. Keep the collector tube (tube n°2) that contains the DNA extract and remove the column. The purified sample is stable for several months at -20 ° C.

Deposit 5 µl for each PCR analysis.

Calculation of the Z factor

For this protocol, the Z factor is calculated as follows:

- **Extraction and purification step**: S1 solution is composed of 1.1 ml of liquid and 0.2 ml of Chelex. Only 1.0 ml of S1 supernatant is purified out of a total of 1.1 ml of S1.
  Thus, the fraction purified is 1/1.1 of the sample. In our equation, Z_1 equals 1.1.

- **Concentration on column step**: 100 µl of solution out of the 200 µl are concentrated on the column and are then eluted in 30 µl.
  Thus, the fraction concentrated is 100/200 of the sample. In our equation, Z_2 equals 2.

- **PCR analysis step**: after the elution step, the microtube contains 30 µl of DNA extract. Only 5 µl are analyzed by PCR.
  Thus, the fraction analyzed is 5/30. In our equation, Z_2 equals 6.

Therefore, the Z factor for the **DNA PURE-FLASH** extraction method is:

$$Z = Z_1 \times Z_2 \times Z_3 = 1.1 \times 2 \times 6 = 13.2.$$

Standard protocol for 'experienced operators' (clear water)

Steps 1 to 5 and 7 to 15 are strictly identical to the conventional protocol. Only step 6 is different:

6. The supernatant contains the extracted DNA. Pipet the entire volume of supernatant, i.e. 1.1 ml (be careful not to take the resin) into a new 2.0 ml microtube.
Note: if the resin of the lysis solution (S1) is pipetted, re-pipet the supernatant into the tube and re-centrifuge.

The remainder of the protocol can be carried out according to the conventional protocol.

Calculation of the Z factor

For this protocol, the Z factor is calculated the same way as for the conventional protocol. Only Z_1 changes:

- **Extraction and purification step**: S1 solution is composed of 1.1 ml of liquid and 0.2 ml of Chelex. All the supernatant is purified.
  Thus, the fraction purified is 1.

Therefore, the Z factor for the **DNA PURE-FLASH** extraction method is: $Z = Z_1 \times Z_2 = 1 \times 40 = 40$.

Conventional protocol for dirty water containing large amounts of suspended particles

Steps 1 to 5 and 7 to 15 are strictly identical to the conventional protocol. Only step 6 is different:

6. The supernatant contains the extracted DNA. Pipet 800 µl of supernatant (taking care not to remove resin) into a new 2.0 ml microtube.
Note: if the resin of the lysis solution (S1) is pipetted, re-pipet the supernatant into the tube and re-centrifuge.

It is advisable to carry out the protocol following the conventional protocol.

Calculation of the Z factor

For this protocol, the Z factor is calculated the same way as for the conventional protocol. Only Z_1 changes:

- **Extraction and purification step**: S1 solution is composed of 1.1 ml of liquid and 0.2 ml of Chelex. Only 0.8 ml of S1 supernatant is purified out of a total of 1.1 ml of S1.
  Thus, the fraction purified is 0.8/1.1 of the sample. In our equation, Z_1 equals 1.375.

Therefore, the Z factor for the **DNA PURE-FLASH** extraction method is: $Z = Z_1 \times Z_2 = 1.375 \times 40 = 55$.