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# Metagenomic DNA Isolation Kit for Water

Cat. No. MGD08420

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## 1. Introduction

The Metagenomic DNA Isolation Kit for Water is designed to isolate randomly-sheared, high molecular weight (HMW) metagenomic DNA directly from unculturable or difficult to culture microbial species present in environmental water samples. The majority of the randomly-sheared DNA isolated using this kit is approximately 40 kb in size and is ready for immediate use in end-repair reactions and subsequent cloning into fosmid vectors. The protocol streamlines the steps in the standard fosmid library construction process by eliminating the need for agarose plug DNA extraction methods, mechanical shearing, and size-selection of the DNA.

For increased reliability and simplicity, we recommend using the isolated DNA in conjunction with Epicentre's CopyControl™ Fosmid or CopyControl HTP Fosmid Library Production Kits.

## 2. Kit Contents

| Desc. | Concentration | Quantity |
|---|---|---|
| **Metagenomic DNA Isolation Kit for Water Contents** | | |
| The Metagenomic DNA Isolation Kit for Water provides sufficient reagents for DNA isolation from 20 filter samples: | | |
| Filter Wash Buffer | | 20 ml |
| Meta-Lysis Solution (2X) | | 6 ml |
| MPC Protein Precipitation Reagent | | 7 ml |
| Proteinase K | (50 µg/µl) | 20 µl |
| Fosmid Control DNA | (100 ng/µl) | 20 µl |
| RNase A | (5 µg/µl) | 20 µl |
| Ready-Lyse™ Lysozyme Solution | | 40 µl |
| TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA) | | 7 ml |

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Required materials not supplied in the kit:

Presterilized 0.45-µm filter
(e.g. Millipore Microfil® V, 47-mm diameter filter or Nalgene 150-ml analytical filter unit)

Miracloth filtration material (Calbiochem) or sterile cheesecloth

1.7-ml microcentrifuge tubes

Tween® 20 (molecular biological grade)

Isopropanol

70% ethanol (made from absolute ethanol)

## 3. Product Specifications

**Storage:** Store the Proteinase K, Ready-Lyse™ Lysozyme, RNase A, and Fosmid Control DNA at –20°C in a freezer without a defrost cycle. Store the remainder of the kit components at room temperature. Note: the Meta-Lysis Solution (2X) may form a precipitate during shipment or storage. If this occurs, heat the bottle at 37°C until the precipitate dissolves. Mix thoroughly and cool to room temperature.

**Storage Buffer:** Proteinase K and Ready-Lyse Lysozyme Solution are supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 10 mM $CaCl_2$, 0.1% Triton® X-100, and 1 mM dithiothreitol; RNase A is supplied in a 50% glycerol solution containing 25 mM sodium acetate (pH 4.6).

**Quality Control:** The Metagenomic DNA Isolation Kit for Water is function-tested by purifying DNA from known concentrations of diluted *E. coli* in a water sample. The size of the DNA is compared with a 40-kb linear control DNA on a 1% agarose gel. DNA quality is assayed by PCR.

## 4. Related Products

The following products are also available:

- CopyControl™ HTP Fosmid Library Production Kit
- CopyControl™ Fosmid Library Production Kit
- CopyControl™ Fosmid Autoinduction Solution
- Direct Lysis Fosmid96 DNA Isolation Kit
- End-It™ DNA End-Repair Kit

## 5. Protocol: Isolation of HMW DNA from Water

1. To remove debris from the collected water sample, pour the water through Miracloth filtration material (Calbiochem), sterile cheesecloth, or centrifuge at 1,000 x g for 5 minutes and collect the water in a sterile container.
2. Filter the water (100 ml) through a presterilized 0.45-µm filter (e.g. Millipore Microfil® V, 47-mm diameter filter or Nalgene 150-ml analytical filter unit) to trap cells on the filter.
3. Using forceps and scissors presoaked in 70% ethanol, remove the membrane from the filter apparatus, cut the membrane into four pieces and place them along the side (near the bottom) of a 50-ml sterile conical tube. The upper surface of the filter needs to face the center (not wall) of the tube. Do not allow the filter membrane to dry out.
4. Prepare the Filter Wash Buffer by adding 2 µl of Tween 20 to 1 ml of Filter Wash Buffer immediately before use. Add 1 ml of Filter Wash Buffer containing 0.2% Tween 20 to the filter pieces in the tube to wash off the microbes trapped on the membrane.
5. Vortex the tube at a low setting to rewet the filter pieces; then increase the setting to the highest speed for ~2 minutes with intermittent breaks.
6. Transfer the cell suspension to a clean microcentrifuge tube, then centrifuge the tube at 14,000 x g for 2 minutes to pellet the cells. Discard the supernatant.
7. Resuspend the cell pellet in 300 µl of TE Buffer, then add 2 µl of Ready-Lyse Lysozyme Solution and 1 µl of RNase A to the cell suspension. Mix by vortexing.
8. Incubate at 37°C for 30 minutes.
9. Add 300 µl of Meta-Lysis Solution (2X) and 1 µl of Proteinase K to the tube. Mix by vortexing.

10. Briefly pulse-centrifuge the tube to ensure that all of the solution is in the bottom of the tube.
11. Incubate at 65°C for 15 minutes.
12. Cool to room temperature, then place on ice for 3-5 minutes.
13. Add 350 µl of MPC Protein Precipitation Reagent to the tube and mix by vortexing vigorously for 10 seconds.
14. Pellet the debris by centrifugation for 10 minutes at 14,000 x g in a microcentrifuge at 4°C.
15. Transfer the supernatant to a clean 1.7 ml microcentrifuge tube and discard the pellet.
16. Add 570 µl of isopropanol to the supernatant. Mix by inverting the tube multiple times.
17. Pellet the DNA by centrifugation for 10 minutes at 14,000 x g in a microcentrifuge at 4°C.
18. Use a pipet tip to remove the isopropanol without dislodging the DNA pellet. Briefly pulse-centrifuge the sample and remove any residual liquid with a pipet tip, without disturbing the pellet.
19. Add 500 µl of 70% ethanol to the pellet without disturbing the pellet. Then centrifuge for 10 minutes at 14,000 x g in a microcentrifuge at 4°C.
20. Use a pipet tip to remove the ethanol without dislodging the DNA pellet. Briefly pulse-centrifuge the sample and remove any residual liquid with a pipet tip, without disturbing the pellet..
21. Air-dry the pellet for 8 minutes at room temperature.

    ***Note:*** *Do not over-dry the pellet.*
22. Resuspend the DNA pellet in 50 µl of TE Buffer.
23. Validate the size and concentration of the isolated DNA by comparison to the Fosmid Control DNA (40 kb; 100 ng/µl) provided in the kit, via gel electrophoresis on a 1% agarose gel. Use 2 µl of the isolated DNA preparation for this analysis.

The isolated HMW DNA is ready for use in end-repair reactions and subsequent cloning into a vector for construction of a fosmid library. If using Epicentre's CopyControl Fosmid Library Production Kit, proceed with one of the following two options:

**Option 1**, End-repair followed by ethanol precipitation to clean up the sample.

1. Thaw and thoroughly mix all of the reagents listed below before dispensing; place on ice.

   Combine the following on ice:

| | | |
|---|---|---|
| x | µl | sterile water |
| 8 | µl | 10X End-Repair Buffer |
| 8 | µl | 2.5 mM dNTP Mix |
| 8 | µl | 10 mM ATP |
| x | µl | Metagenomic DNA (from step 22, above) |
| 4 | µl | End-Repair Enzyme Mix |
| 80 | µl | Total reaction volume |

2. Incubate at room temperature for 45 minutes.
3. Incubate at 70°C for 10 minutes to inactivate the End-Repair Enzyme Mix.
4. Precipitate the DNA.
   a) Add 1/10 volume of 3 M Sodium Acetate (pH 7.0) and mix gently.
   b) Add 2.5 volumes of ethanol. Cap the tube and mix by gentle inversion.
   c) Allow the DNA to precipitate for 10 minutes at room temperature.
   d) Centrifuge the precipitated DNA for 20 minutes in a microcentrifuge, at ≥10,000 x g.
   e) Carefully aspirate the supernatant from the pelleted DNA.
   f) Wash the pellet gently with cold, 70% ethanol using care not to disrupt the DNA pellet; repeat steps d) and e).
   g) Remove the excess alcohol by a quick centrifugation followed by aspiration with pipet tip.
   h) Air-dry the pellet for 5-10 minutes (longer dry times will make resuspension of the DNA difficult).
5. Gently resuspend the DNA pellet in 6-8 µl of TE Buffer.
6. Proceed to Ligation (Part E) of the CopyControl Fosmid Library Production Kit protocol.

**Option 2**, Perform steps 1-3 above for End-Repair followed by gel purification of the DNA on a 1% agarose gel. After gel purification, proceed to Ligation (Section E) of the CopyControl Fosmid Library Production Kit protocol.



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