Microzone Product Datasheet

Product code: 2C2S-50 Lot Number:

# Cell2Seq - 16S rRNA Microbial Identification Kit

| P. Code | Size in 50 Rxn | Component | Description | Lot Number | Expiry |
|---|---|---|---|---|---|
| 2MLP-1 | 1 x 1 mL | microLYSIS Plus | Direct to PCR, DNA Release Buffer. | | |
| 2MMC-1.25 | 1 x 0.625 mL | 2X MegaMix Crystal Q5 High Fidelity Mastermix | Specially optimised reaction buffer containing Q5 HotStart High Fidelity Polymerase, dNTPs, $MgSO_4$ and enhancers. | | |
| 2PM16SF-0.35 | 1 x 0.35 mL | 16S rRNA Forward Primer | Optimal concentration of forward primer ready-to-use. | | |
| 2PM16SR-0.35 | 1 x 0.35 mL | 16S rRNA Reverse Primer | Optimal concentration of reverse primer ready-to-use. | | |
| 2MCL-1.25 | 1 x 1.25 mL | microCLEAN | PCR Clean-Up. | | |
| 5JWA-1 | 1 x 1 mL | Just Water (Molecular Grade Water) | Aliquoted, quality controlled, nuclease-free, molecular grade water. | | |

## Applications

- Sanger Sequencing sample preparation workflow: microbial lysis, high-fidelity amplification and PCR clean-up
- Classify microorganisms**,** construct phylogenetic trees, uncover evolutionary relationships, detect novel species and more

## Product Description

Part of the 'Free From' Microzone range, Cell2Seq – 16S rRNA Microbial Identification Kit is a cell-to-sequence solution designed to streamline and simplify the 16S Sanger sequencing workflow. This innovative kit provides all the necessary components for the three critical steps for sample preparation for sequencing: microbial lysis, high-fidelity PCR amplification, and post-PCR clean-up.

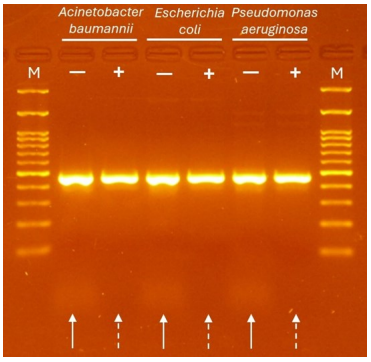
Microbial lysis is achieved with microLYSIS Plus, a reagent formulated for efficient microbial lysis and high-quality DNA extraction through a rapid thermal lysis protocol. The whole process takes place in a single tube and does not involve the use of solvents or other harmful chemicals usually required in traditional DNA extraction methods.

High-fidelity PCR amplification is performed using the 2X MegaMix Crystal Q5 High Fidelity Mastermix, which consists of Q5 DNA Polymerase in an enhanced buffer with optimal $Mg^{2+}$ concentration, balanced dNTPs, and a proprietary formulation of stabilisers and enhancers specifically optimised for our enzyme. Q5 High Fidelity DNA Polymerase offers unmatched accuracy with a 280-fold higher fidelity compared to Taq Polymerase and a 5-fold higher fidelity compared to standard Pfu Polymerase. This enzyme features a Hot-Start mechanism for increased specificity and sensitivity, allowing for room temperature reaction setup.

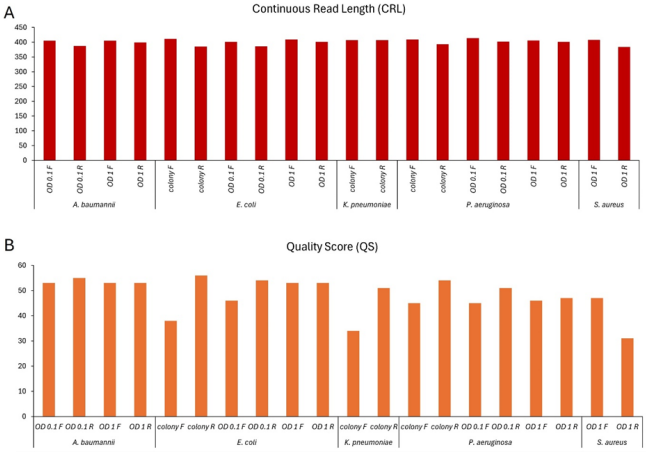
Post-PCR clean-up utilises microCLEAN reagent, which requires less than 15 minutes to efficiently remove unwanted residual dNTPs, primer-dimers, enzymes and unincorporated dyes from PCR products while maximising amplicon recovery for high-quality sequencing. It is a quick and simple protocol with minimal requirements for equipment and plasticware, significantly reducing the environmental impact of the process.

## Key Features

- Microbial lysis, high-fidelity PCR amplification, and effective post-PCR clean-up steps in a single kit
- Significantly reduced processing time without compromising sequencing results
- Robust, rapid, and environmentally conscious workflow
- Accurate and reliable microbial identification using Sanger sequencing.




**Successful amplification from bacterial lysates in microLYSIS Plus and no sample loss after PCR clean-up step using Cell2Seq – 16S rRNA Microbial Identification Kit.** V3-V4 regions of 16S rRNA gene amplified with 2X MegaMix Crystal Q5 HiFi MasterMix: before (-) or after PCR clean-up with microCLEAN (+). Solid arrows show the presence of PCR residues; Dashed arrows show removal of PCR residues after microCLEAN PCR clean-up. M = 100 bp DNA ladder.




**Excellent quality metrics obtained from 16S sequencing using Cell2Seq – 16S rRNA Microbial Identification Kit.** Different bacteria species prepared in microLYSIS Plus using either a bacterial colony or broth (OD of 0.1 or OD 1), 16S amplification was performed with MegaMix Crystal Q5 High-Fidelity MasterMix and PCR clean-up was achieved with microCLEAN. Graphs show the contiguous read length (A), and Quality Score (B) obtained from Sanger sequencing.

**Product Handling and Storage**

To ensure the quality of the product until the expiry date keep at the recommended storage temperature and limit exposure to light.

**Licenses**

This product is covered by one or more patents.

This product is licensed for research and commercial use from Bio-Rad Laboratories, Inc., under U.S. Pat. Nos. 6,627,424, 7,541,170, 7,670,808, 7,666,645, and corresponding patents in other countries. No rights are granted for use of the product for Digital PCR or real-time PCR applications, with the exception of quantification in Next Generation Sequencing workflows.

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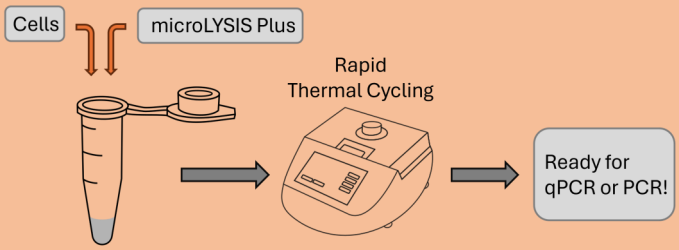
## Step 1: Rapid Lysis




### Protocol

Follow guidelines for the addition of cells to microLYSIS Plus:

For colonies – pick a colony and add to 20 µL of microLYSIS Plus, ensure the colony is removed from the loop before mixing well.

For broths – add 2 µL of broth (up to OD of 1) to 20 µL microLYSIS Plus and mix well.

### Thermal cycling – Lysis

Overlay with mineral oil if necessary. Place in a Thermal Cycler and set profile as follows:

| Step | Profile for most cell types | Profile for difficult-to-lyse cells |
|---|---|---|
| 1 | 75°C for 5 mins | 65°C for 15 mins |
| 2 | 95°C for 2 mins | 96°C for 2 mins |
| 3 | | 65°C for 4 mins |
| 4 | | 96°C for 1 mins |
| 5 | | 65°C for 1 mins |
| 6 | | 96°C for 30 secs |

After lysis, the lysate can be added directly to PCR as template. Alternatively, it can be stored at -20°C for later use.

Samples from microLYSIS PLUS can be used in end-point PCR, qPCR and High Fidelity PCR reactions.

## Step 2: High-Fidelity Amplification

Thaw all reagents completely and mix well before use.

Prepare a master mix as described in the table below.

| Components | Volume |
|---|---|
| 2X MegaMix Crystal Q5 HiFi MasterMix | 12.5 µL |
| 16s rRNA Forward Primer (5 µM) | 1.25 µL |
| 16s rRNA Reverse Primer (5 µM) | 1.25 µL |
| Template | 1.5 µL |
| Just Water (Molecular Grade Water) | 8.5 µL |

Mix gently, avoiding bubbles, centrifuge if necessary.

### Thermocycling

The following general cycling conditions are intended for use as a guide and can vary depending on the template being used.

| Step | Cycles | Temperature | Time |
|---|---|---|---|
| Initial Denaturation | 1 | 98°C | 30 seconds |
| Denaturation | | 98°C | 5 seconds |
| Annealing | 25 - 27 | 55°C | 10 seconds |
| Elongation | | 72°C | 30 seconds |
| Final Elongation | 1 | 72°C | 2 min |

For Research Use Only. Not for use in diagnostic procedures.

## Step 3: PCR Clean-Up

1. Add 25 µL of microCLEAN to the 25 µL PCR product in a microcentrifuge tube or plate well.
2. Mix well by pipetting up and down or pulse vortexing.
   **Note:** A 5 minutes incubation may increase yield recovery.

### For microcentrifuge tubes:

3. Centrifuge at 13,000 ×g for 7 minutes.
   **Note:** Centrifugation will result in an invisible pellet.
4. Remove supernatant.
   **Tip:** Avoid touching the tube walls to not disturb the pellet.
5. Re-centrifuge briefly and remove any remaining liquid.
   **Tip:** Use a P20 pipette to guarantee complete removal of liquid.
6. Resuspend pellet in Molecular Grade Water (such as Microzone's Just Water) or Tris buffer by pipetting or pulse vortexing.
   **Tip:** Resuspend in same volume as sample input (25 µL) or reduce volume if concentrated PCR product is desired.
7. Incubate at room temperature for 5 minutes to rehydrate DNA.
8. Purified PCR product is ready for sequencing or storage.

### For plates:

3. Centrifuge at 2000 to 4000 ×g for 40 minutes.
4. Place plate upside down on to tissue paper in the centrifuge.
5. Pulse centrifuge to < 40 ×g for 30 seconds to remove all liquid.
   **Note:** Centrifugation will result in an invisible pellet.
6. Resuspend pellet in Molecular Grade Water (such as Microzone's Just Water) or Tris buffer by pipetting or pulse vortexing.
   **Tip:** Resuspend in same volume as sample input or reduce volume if concentrated PCR product is desired.
7. Incubate at room temperature for 5 minutes to rehydrate DNA.
8. Purified PCR product is ready for sequencing or storage.

## Sanger Sequencing Sample Preparation

This protocol is intended for use as a guide; amount of PCR product and primers may change depending on sequencer or servicer used.

Cell2Seq Kit provides enough forward and reverse primers for 50 sequencing reactions each, considering the following protocol:

1. Add 5 µL of purified PCR product and 5 µL of the forward primer (5 µM) in a centrifuge tube.
2. Add 5 µL of purified PCR product and 5 µL of the reverse primer (5 µM) in another centrifuge tube.
3. Send samples to Sanger Sequencing or add 10 µL of the preferrable Dye Terminator Cycle Sequencing Mix (not included with the Cell2Seq - 16S rRNA Microbial Identification Kit).

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