



LysoPrep Lyo Lysozyme Pretreatment Kit for DNA Extraction manual

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LysoPrep Lyo reagent kit is intended for pre-treating human biological material and bacterial cultures with lysozyme before DNA extraction. This procedure increases the yield of bacterial DNA and reduces the inhibitor concentration.

Lysozyme destroys peptidoglycans, which are part of the cell walls of microorganisms, due to the hydrolysis of N-glycosidic bonds. Also, when incorporated into the cytoplasmic membrane, lysozyme molecules lead to its damage and the formation of pores, ultimately destroying bacterial cells.

The kit is ready to use. One kit is designed to process 50 samples.

Kit components

Kit component	Count
	21133 50 assays
12315, Lysozyme, 20 mg	5
A6850, Lysozyme dilution Buffer, 200 uL	5

Store at temperature between -22 °C and 8 °C. Transport at temperature between 0 °C and 25 °C up to 10 days.

Shelf life 12 months.

More detailed storage conditions for the kit components are given below.

Required equipment and materials not included in the kit:

- Microcentrifuge-vortex
- Centrifuge for 1.5 mL tubes with an RCF of at least 14,000× *g*
- Solid-state thermostat that maintains temperatures up to 65 °C
- 1.5 mL microcentrifuge tubes with caps, RNase- and DNase-free
- Disposable filter tips, DNase- and RNase-free, volumes 20 µL, 200 µL, 1000 µL
- Container for discarding used tips, tubes, and other consumables
- Sterile saline solution
- Glycerol (if necessary)

Sample preparation

The samples of human stool (feces, including meconium) and bacterial cultures can be used for the analysis. To achieve maximum quality, keep the following general recommendations:

- To achieve accurate results, the quality of the collection of the biomaterial sample and its proper storage, transportation, and pre-processing are crucial.
- Incorrect collection of biomaterials may cause distorted results and, therefore, the need for repeated sampling.
- At the biomaterial preparation stage, filter tips that do not contain RNase and DNase should be used.
- When adding a lysozyme solution into a test tube with biological material, do it carefully, avoiding contact of the tip with the inner walls of the test tube. If contact occurs, replace the tip.
- The tip should be changed each time the solution is removed from the sample.
- To prevent contamination, keep open the cap of only the test tube you are working on (adding the sample/reagent, removing the supernatant); after adding all the necessary reagents, close the tube and only after open the next one.

1. Collecting the feces

Use samples of feces or meconium weighing (volume) approximately 1.0–3.0 g (1.0–3.0 mL) for analysis. Transfer approximately 1.0 g of the sample into a sterile dry vial with a separate filter tip or disposable spatula. After collecting the feces, close the vial tightly and label it.

Important! Before DNA extraction, preliminary processing of samples of biological material is required (see the step *Preparation of feces and meconium samples*).

2. Collecting the bacteria

Use a disposable microbiological loop or spatula to take biomaterial from liquid or solid media. Place a single colony of cells or 100 μL of liquid medium into a 1.5-2.0 mL disposable tube pre-filled with 500 μL of sterile saline solution. After the material is collected, cap the tube tightly and label it.

3. Transportation and storage of samples for research

Fecal or meconium samples can be transported and stored:

- at room temperature (from 18 °C to 25 °C) — no more than 6 hours;
- at temperatures from 2 °C to 8 °C — no more than 3 days.

If it is necessary to store the material for more than a day or for a long time, preparing a fecal suspension with the addition of glycerol is recommended.

Bacterial cultures can be transported and stored:

- at temperatures from 2 °C to 8 °C — no more than one day;
- at temperatures from -18 °C to -20 °C — no more than one week;
- at a temperature of -70 °C — for a long time.

Important! Only one-time freezing and thawing of the material is allowed.

4. Preparation of feces and meconium samples (preparation of suspension)

1. Transfer approximately 0.1-0.2 g (mL) of feces into a 1.5 mL plastic tube containing 1.0 mL sterile saline solution.
2. Thoroughly mix the contents of the tube for 5-10 s using a microcentrifuge-vortex.
3. If it is impossible to analyze the sample within one day or long-term storage is necessary, add glycerol to feces suspension to a final concentration of 10–15%.
4. Thoroughly mix the sample with glycerol and stand it for 30–40 min to ensure complete homogenization before freezing.
5. Fecal suspension with glycerol can be transported and stored as follows:
 - at a temperature of -20 °C — for 1 week;
 - at a temperature of -70 °C — for a long time.

Preparing kit components

Lysozyme from the **LysoPrep Lyo** kit must first be dissolved:

1. Add 200 μ L of buffer to a tube containing lyophilized lysozyme.
2. Shake the tube on a microcentrifuge-vortex for 3-5 seconds until the crystals dissolve entirely.
3. Close the tube tightly.
4. Sediment the drops from the lid for 1-3 seconds in a microcentrifuge-vortex.
5. The obtained volume of lysozyme solution is sufficient to process 10 samples, including negative control.

The unused rest of the prepared lysozyme solution can be stored at -18 °C to

-22 °C for the kit expiration date.

Important! Only one cycle of freezing/thawing of the solution is allowed.

Sample processing

1. General recommendations

- When handling samples, it is recommended to use disposable RNase- and DNase-free filter tips.
- When adding reagents into a tube with biological material, do it carefully, avoiding contact of the tip with the inner walls of the test tube. If contact occurs, replace the tip.
- Test samples and negative control «NC» must be processed simultaneously.

2. Treatment of fecal suspension

1. Centrifuge the tubes with fecal suspension at $13,000 \times g$ for 30 seconds at room temperature to settle debris to the bottom of the tube.
2. Label each 1.5 mL disposable tube for each sample to be tested and for the negative control «NC».

Important! To prevent contamination, keep open the cap of only the test tube you are working on (adding the sample/reagent, removing the supernatant); after adding all the necessary reagents, close the tube and only after open the next one. It is not allowed to work simultaneously with several test tubes with open lids.

3. Transfer 100 μ L of the middle fraction from the fecal suspension tubes to the test tubes. The sample should not be added to the «NC» tube.
4. Add 100 μ L of sterile saline or elution buffer from the Nucleic Acid Isolation Reagent Kit into the «NC» tube.
5. Add 20 μ L of lysozyme solution to the labeled sample tubes and to the «NC» tube.

6. Gently shake the tubes in a microcentrifuge-vortex for 3-5 seconds.
7. Centrifuge the tubes for 1-3 seconds to release droplets.
8. Incubate samples with lysozyme for 60 minutes at room temperature or 30 minutes at 37 °C. During incubation, gently shake the tubes 2-3 times for 3-5 seconds in a microcentrifuge-vortex.
9. Centrifuge the tubes in a microcentrifuge-vortex for 60 seconds.

3. Treatment of bacterial culture

1. Label 1.5 mL disposable tube for each test sample and negative control «NC».
2. Add 100 µL of bacterial suspension to each tube. The sample should not be added to the «NC» tube.
3. Add 100 µL of sterile saline or elution buffer from the Nucleic Acid Isolation Reagent Kit into the «NC» tube.
4. Add 20 µL of lysozyme solution to the test and «NC» tubes.
5. Gently shake the tubes in a microcentrifuge-vortex for 3-5 seconds.
6. Centrifuge the tubes for 1-3 seconds to release droplets.
7. Incubate samples with lysozyme for 60 minutes at room temperature or 30 minutes at 37 °C. During incubation, gently shake the tubes 2-3 times for 3-5 seconds in a microcentrifuge-vortex.
8. Centrifuge the tubes in a microcentrifuge-vortex for 60 seconds.

DNA extraction

Samples treated with lysozyme using the **LysoPrep Lyo** kit should be used immediately for DNA extraction.

Utilize the entire volume (120 μ L) of the pretreated sample for DNA extraction following the instructions for the nucleic acid isolation kit in use.

Use our **LumiMag UNI**, **LumiMag FEC**, **LumiPure UNI**, or **LumiSpin UNI** Nucleic Acid Isolation kits for the best DNA extraction.

Best before date

The shelf life of the kit is 12 months from the date of acceptance by the manufacturer's quality control department, subject to all transportation, storage, and operation conditions. Expired reagent kits cannot be used.

Storage kit components

- Kit components should be stored at $-22\text{ }^{\circ}\text{C}$ to $+8\text{ }^{\circ}\text{C}$ for the entire shelf life of the reagent kit.
- The prepared lysozyme solution should be stored in a freezer at a temperature between $-18\text{ }^{\circ}\text{C}$ and $-22\text{ }^{\circ}\text{C}$, with the possibility of defrosting once.
- Kit components stored violating the regulated regime are not subject to use.



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