

Geneaid™ DNA Isolation Kit (Bacteria) Quick Protocol

For research use only

Catalogue Number

GEE005, GEE150, GEE1.5K, GEE1.5K+,

Instruction Manual Download

When using this product for the first time, or if you are unfamiliar with the procedure, please scan the QR code and download the complete instruction manual.

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0.5-1.5 x 10⁹ Bacteria Protocol Procedure

1. Sample Preparation

Gram (-) Bacteria

Transfer the **Gram (-) negative bacteria culture (0.5-1.5 x 10⁹)** to a 1.5 ml microcentrifuge tube then centrifuge at 14-16,000 x g for 1 minute. Discard the supernatant then proceed to step 2 Lysis.

Gram (+) Bacteria

Transfer the **Gram (+) bacteria culture (0.5-1.5 x 10⁹)** to a 1.5 ml microcentrifuge tube. Centrifuge for 1 minute at 14-16,000 x g then discard the supernatant. Transfer the required volume of **Gram+ Buffer (100 µl/sample)** to a 15 ml centrifuge tube. Add **Lysozyme (0.8 mg/100 µl)** to **Gram+ Buffer** (in the 15 ml centrifuge tube) then vortex to completely dissolve the Lysozyme. Transfer **100 µl of Gram+ Buffer** (make sure Lysozyme was added) to the bacteria pellet. Resuspend the pellet by shaking vigorously or pipette. Incubate at room temperature for 10-20 minutes. During incubation, invert the tube every 2-3 minutes.

2. Lysis

Add **300 µl of Cell Lysis Buffer** to the tube then mix by vortex. Incubate at 60°C for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step

Following 60°C incubation, add 1.5 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 5 minutes.

3. Protein Removal

Add **100 µl of Protein Removal Buffer** to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 14-16,000 x g for 3 minutes to form a tight pellet.

NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 14-16,000 x g for another 3 minutes.

4. DNA Precipitation

Transfer the supernatant to a clean 1.5 ml microcentrifuge tube then add **300 µl of isopropanol** and mix well by gently inverting 20 times. Centrifuge at 14-16,000 x g for 5 minutes then carefully discard the supernatant and add **300 µl of 70% ethanol** to wash the pellet. Centrifuge at 14-16,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add **100 µl of DNA Hydration Buffer** then gently vortex for 10 seconds. Incubate at 60°C for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

Components

Geneaid™ Bacteria Kit	GEE005	GEE150	GEE1.5K	GEE1.5K+
Number of cells processed per kit	5 x 10 ⁹	1.5 x 10 ¹¹	1.5 x 10 ¹²	1.5 x 10 ¹²
Cell Lysis Buffer	3 ml	100 ml	1000 ml	1000 ml
Protein Removal Buffer	1 ml	40 ml	400 ml	400 ml
DNA Hydration Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	1 ml	50 ml	500 ml	500 ml
Gram+ Buffer	1.5 ml	30 ml	150 ml	150 ml
RNase A (10 mg/ml)	25 µl	550 µl	Not included	5 ml
Lysozyme	8 mg	130 mg	Not included	610 mg x 2

Storage

Cell Lysis Buffer, Protein Removal Buffer, DNA Hydration Buffer, Gram+ Buffer should be stored dry at room temperature (15-25°C) for up to 2 years. RNase A should be stored at 4°C for extended periods. Lysozyme should be stored at -20°C for extended periods.