

GeneJET Genomic DNA Purification Kit from Thermo Fisher Scientific

- ◆ Protocol for gram-negative bacteria.
- ◆ Harvest up to 2×10^9 bacterial cells in a 1.5 or 2 mL microcentrifuge tube by centrifuging for 10 minutes at 5,000 x *g*. Discard the supernatant.
- ◆ Resuspend the pellet in 180 μ L of **Digestion Solution**. Add 20 μ L of **Proteinase K Solution** and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
- ◆ Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (~30 minutes).
- ◆ Add 20 μ L of **RNase A Solution**, mix by vortexing and incubate the mixture for 10 minutes at room temperature.
- ◆ Add 200 μ L of **Lysis Solution** to the sample. Mix thoroughly by vortexing for about 15 seconds until a homogeneous mixture is obtained.
- ◆ Add 400 μ L of **50 % ethanol** and mix by pipetting or vortexing.
- ◆ Transfer the prepared lysate to a **GeneJET Genomic DNA Purification Column** inserted in a collection tube. Centrifuge the column for 1 min at 6,000 x *g*. Discard the collection tube containing the flow through solution. Place the **GeneJET Genomic DNA Purification Column** into a new 2 mL collection tube.
- ◆ Add 500 μ L of **Wash Buffer I**. Centrifuge for 1 minute at 8,000 x *g*. Discard the flow through and place the purification column back into the collection tube.
- ◆ Add 500 μ L of **Wash Buffer II** to the **GeneJET Genomic DNA Purification Column**. Centrifuge for 3 minutes at maximum speed. Discard the collection tube containing the flow through solution and transfer the **GeneJET Genomic DNA Purification Column** to a sterile 1.5 mL microcentrifuge tube.

- ◆ Add 200 µL of **Elution Buffer** to the centre of the **GeneJET Genomic DNA Purification Column** membrane to elute genomic DNA. Incubate for 2 minutes at room temperature and centrifuge for 1 minute at 8,000 x *g*.
- ◆ Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

From: [Thermo Fisher Scientific](#)