

MasterPure™ Gram Positive DNA Purification Kit

1. What is the MasterPure Gram Positive DNA Purification Kit, and how does it work?

The MasterPure Gram Positive DNA is designed for rapid, high-yield purification of DNA from Gram positive bacterial species. The kit includes Ready-Lyse™ Lysozyme, a recombinant enzyme with high specific activity that facilitates lysis of Gram positive bacteria. The procedure provides high yields of pure, intact bacterial DNA without bead-beating, spin columns, or toxic chemicals (Figure 1).

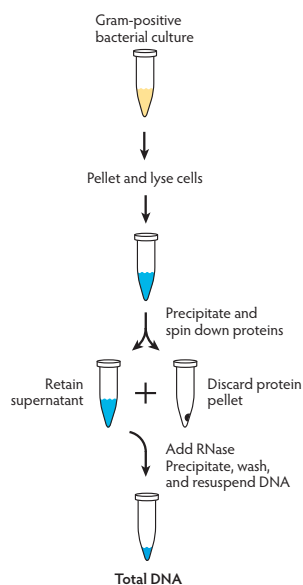


Figure 1. The MasterPure Gram Positive DNA Purification Kit provides high yields of pure total DNA.

2. What kinds of samples can I use with the MasterPure Gram Positive Kit?

The kits can be used with a variety of bacteria, including *Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, and many other species.

3. For what applications can I use the DNA purified with the MasterPure Gram Positive Kit?

The purified bacterial DNA is suitable for a variety of molecular biology applications, such as cloning, PCR amplification, Southern blotting, genomic library preparation, and next gen sequencing (NGS). Researchers have published many novel applications for the MasterPure Gram Positive DNA Purification Kit, including: quality validation of commercial probiotics by NGS [1]; NGS-based mechanistic studies of multidrug-resistant *Klebsiella pneumoniae* [2]; identification of a novel bacterial species in ground beef by capillary sequencing [3]; and developing a model system to study periodontal disease using qPCR in a multispecies biofilm [4]. In addition, the kit has been used to purify DNA for obtaining draft and complete genomes of a range of bacterial strains and serotypes, including two *Propionibacterium acnes* strains in patients with skin diseases [5].

FAQs

4. I work with both Gram positive and Gram negative bacteria. Can I use the MasterPure Gram Positive DNA Purification Kit for both types?

Due to the relatively thick cell wall and peptidoglycan layer, Gram positive bacteria can be difficult to lyse by ordinary methods. The MasterPure Gram Positive Kit procedure includes a special lysis step, using Ready-Lyse Lysozyme that is performed for 30 minutes to overnight, depending on the species, before protein removal and DNA precipitation. However, the kit will also work with Gram negative bacteria, so you can use the same kit for both types of bacteria.

5. How much starting material do I need for the MasterPure Gram Positive DNA Purification Kit? How much DNA will I get after purification?

The kit protocol can be scaled proportionally, depending on the volume of liquid culture harvested and the growth conditions. The protocol provided in the kit is based on starting with 1 mL of an overnight Gram Positive bacterial culture. The final yield of DNA will vary, depending on the bacterial species. Typical DNA yields when starting with a 1 mL overnight culture include 9 µg for *Bacillus subtilis*, 3 µg for *Streptococcus mutans* and *Listeria monocytogenes*, 4 µg for *Staphylococcus epidermidis*, and 8 µg for *Staphylococcus aureus*.

6. Can I use DNA purified with the MasterPure Gram Positive Kit to prepare an NGS library?

Yes, the kit has been used successfully to purify DNA for both NGS and capillary sequencing.

7. What is the best way to quantify DNA purified using the MasterPure Gram Positive Kit?

Spectrophotometric methods (e.g., A_{260}) to quantify DNA or RNA, although in common use, may overestimate the concentration. The best method to quantify DNA is by fluorometry using a DNA-specific dye, such as Hoechst 332581 (bisbenzimidazole), or PicoGreen® dye (Thermo Fisher Scientific). These dyes bind specifically to double-stranded DNA and not to nucleotides, single-stranded DNA, or RNA.

References

1. Lewis ZT et al. 2016. Validating bifidobacterial species and subspecies identity in commercial probiotic products. *Pediatr Res* **79**:445-452.
2. Jana B et al. 2017. The secondary resistome of multidrug-resistant *Klebsiella pneumoniae*. *Sci Rep* **7**:42483.
3. Rooney AP et al. 2011. *Peptoniphilus methionivorax* sp. nov., a Gram Positive anaerobic coccus isolated from retail ground beef. *Intl J Syst Evol Microbiol* **61**:1962-1967.
4. Millhouse E et al. 2014. Development of an in vitro periodontal biofilm model for assessing antimicrobial and host modulatory effects of bioactive molecules. *BMC Oral Health* **14**:80.
5. Petersen R et al. 2015. Draft genome sequences of two *Propionibacterium acnes* strains isolated from progressive macular hypomelanosis lesions of human skin. *Genome Announc* **3**:e01250-15.

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