

# DNA Extraction Using prepGEM™ Bacteria



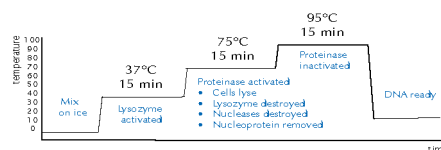
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### General tips

- All manipulations should be performed in a PCR hood to reduce contamination from laboratory sources.
- Workspaces for handling unprocessed samples and PCR products should be kept separate.
- Use only certified DNA-free tubes and reagents. Even the most purified water can contain traces of bacterial DNA.
- Wash any equipment that will come into contact with the sample in 0.05% hypochlorite bleach. Rinse thoroughly with DNA-free water.
- Good laboratory practice and safe handling guidelines should be followed at all times when working with microorganisms.

### Procedure Outline



### Preparation of the Lysozyme

- Lysozyme is provided as a lyophilised powder. To use, resuspend in 100 mM Tris pH 8.0 to the volume specified on the label.
- To reduce the potential for contamination and activity loss, the lysozyme is best stored in small aliquots at -20°C.

2 To order prepGEM™ Bacteria visit [www.zygem.com](http://www.zygem.com) or email: [info@zygem.com](mailto:info@zygem.com) or contact your local distributor

### Colony Stabs

Cells from colonies can be resuspended directly in the extraction buffer.

*Do not lift too much of the colony.*

**NOTE:** For colonies producing large amounts of polysaccharide, pre-washing cells in the **WASH** buffer can improve yields (SEE PAGE 6)

1. For each extraction make up:
  - 88 µl DNA-free water
  - 10 µl of 10x Buffer **GREEN**
  - 1 µl prepGEM™
  - 1 µl lysozyme
2. Lift a small amount of the colony with a sterile loop.
3. Incubate at:
  - 37°C for 15 minutes
  - 75°C for 15 minutes
  - 95°C for 15 minutes

A thermal cycler can be used for this step

The sample is now ready for quantification and analysis.

### Liquid Cultures

1. For each extraction, make up:
  - 68 µl DNA-free water
  - 10 µl of 10x Buffer **GREEN**
  - 1 µl prepGEM™
  - 1 µl lysozyme
2. Add 20 µl of liquid culture
3. Incubate at:
  - 37°C for 15 minutes
  - 75°C for 15 minutes
  - 95°C for 15 minutes

A thermal cycler can be used for this step

The sample is now ready for quantification and analysis.

**TIPS:**

- If more than 20 µl of culture is required, then the cells should be pelleted by centrifugation and resuspended in the extraction cocktail. The recipe for the extraction cocktail should be adjusted appropriately to make a final volume of 100 µl.
- For capsulated bacteria and bacteria that secrete large amounts of polysaccharides, a pre-wash in the wash buffer provided can be added to the procedure (SEE PAGE 6).

## Swabs



1. **WASH** buffer is provided as a 5x solution. Add 1 part buffer to 4 parts water (SEE PAGE 6).
2. Wash the swab for 30 seconds in 0.4 ml of wash buffer by pressing the swab against the side of the tube below the liquid. Before discarding the swab, squeeze the swab head on the wall of the tube to extract as much of the liquid as possible.



3. Sediment the cells by centrifugation at 10,000 r.c.f for 5 minutes.



4. For each extraction, make up:
  - 88 µl DNA-free water
  - 10 µl of 10x Buffer **GREEN**
  - 1 µl *prepGEM*<sup>TM</sup>
  - 1 µl lysozyme



5. Resuspend the cells in this solution and incubate at:
  - 37°C for 15 minutes,
  - 75°C for 15 minutes
  - 95°C for 15 minutes



A thermal cycler can be used for this step

The sample is now ready for quantification and analysis.

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## Workflow Shortcuts

The *prepGEM*<sup>TM</sup> Bacteria kit has been tested on a range of species with different characteristics and morphologies. It should be recognised that no two microorganisms are alike and when non-typical samples are used, the methods provided in the QuickStart Guide can be adapted. Furthermore, the workflows and procedures can be streamlined for many sample types.

The following is a list of shortcuts that may be possible to simplify the workflow without unduly affecting DNA yield.

1. For most sample types, the 95°C step can be reduced to 5 minutes.
2. For some sample types (for example *E. coli*) the lysozyme step is not needed.
3. The 37°C and the 75°C steps may be reduced to 10 minutes for some organisms.

## Optimisation for difficult samples

1. Samples with large amounts of extraneous material (for example pus or mucus) can be centrifuged at 500 r.c.f. for 5 minutes to sediment the debris without pelleting the bacteria. The supernatant should then be transferred to a new tube and the cells pelleted at 10,000 r.c.f for 10 minutes.
2. For more difficult bacteria, extended 37°C and 75°C incubations can be used and/or more *prepGEM*<sup>TM</sup> added.

Check for Application Notes on the ZyGEM website for dealing with different sample types.

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## ZyGEM **WASH** Buffer

The wash buffer is a proprietary formulation designed to reduce problems caused by polysaccharide. For bacteria producing large amounts of polysaccharide, or samples presented in mucus (for example sputum, throat or vaginal swabs) a pre-wash in this buffer is recommended.

In general, this involves the cells (or swab in 0.4 ml of 1x **WASH** Buffer followed by a 5 minute centrifugation at 10,000 r.c.f. The pellet should be drained thoroughly (it is important to remove all of the wash buffer) and then resuspended in 100 µl of the extraction cocktail.

## COLONIES

1. Transfer a loop full of the colony into a 1.6 ml tube and wash the cells in 400 µl of ZyGEM **WASH** buffer.
2. Mix by voortexing or pipetting.
3. Centrifuge the cells at 10,000 r.c.f. for 5 minutes and resuspend in the extraction cocktail.
4. For difficult samples, the wash step can be repeated.

## HIGH POLYSACCHARIDE LIQUID CULTURES AND SPUTUM

1. Pipette 20 - 100 µl of culture / sputum into a 1.5 ml tube and add 400 µl of ZyGEM **WASH** buffer.
2. Vortex vigorously to disperse the cells.
3. Centrifuge the cells at 10,000 r.c.f. for 5 minutes and resuspend in the extraction cocktail.
4. For difficult samples, the wash step can be repeated.

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## Technical tips for sample management

- *prepGEM*<sup>TM</sup> Bacteria is a preparative method for DNA extraction from Gram -ve and Gram +ve bacteria. The *prepGEM*<sup>TM</sup> method lyses cells and removes nucleoproteins from the DNA. Extracted DNA can be used for many types of genotyping including SNP analysis as well as quantitative, multiplex and end-point PCR.
- The wash step is only required for bacteria that produce large amounts of polysaccharide and for swabs from mucus membranes.
- DNA extracted using *prepGEM*<sup>TM</sup> is largely single-stranded because of the 95°C heat step.
- For accurate yield assessment, a qPCR is recommended. The DNA produced by *prepGEM*<sup>TM</sup> is approximately 90% single-stranded. If standard fluorescent chelating dyes are to be used for quantification, then this factor should be taken into consideration.
- As with any preparative method for nucleic acid extraction, best results are obtained when samples are handled at 4°C, or on ice, before and after extraction.
- When storing sample after extraction, centrifuge the sample for 5 minutes at 6000 r.c.f. to remove the debris and aspirate the supernatant from the sedimented debris and store separately.
- The presence of EDTA in the sample can reduce enzyme performance in the extraction. This problem can be overcome by adding CaCl<sub>2</sub> to a final concentration of 200 µM.
- The *prepGEM*<sup>TM</sup> reagents are stable at 4°C but after tubes have been opened and for longer term storage, reagents should be stored at -20°C.

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