

Important Notes

Please read the following notes before starting any of the Genomic Spin procedures.

Before equipment

- **Buffer NL and NCL:** shaking before use.
 - **Note:** If a precipitate has formed, dissolve by incubating at 56°C. Do not add Proteinase K directly to Buffer NL.
- **Add ethanol (96-100%) to Wash C and D Solution,** before use first (see bottle label for volume).
- Equilibrate samples to room temperature (15–25°C).
- Heat a water bath or heating block to 56°C for use in the Mini step.
- Equilibrate Elution Buffer or distilled water to room temperature for elution.
- All centrifugation steps should be carried out at room temperature.
- Use carrier DNA if the sample contains <10,000 genome equivalents.
- Avoid repeated freezing and thawing of stored samples, since this leads to reduced DNA size

Vacuum notes:

- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during manipulations.
- Wear safety glasses when working near a manifold under pressure.
- The vacuum pressure is the pressure differential between the inside of the manifold and the atmosphere (standard atmospheric pressure: 1013 millibar or 760 mmHg) and can be measured using a vacuum regulator.
- Use of a vacuum pressure lower than recommended may reduce DNA yield and purity.

Genomic Cell/Tissue Spin Mini Kit Protocol

Please read “Important Notes” before starting.

1. **Cut up to 25 mg of tissue (up to 10 mg spleen) into small pieces, place in a 1.5 ml microcentrifuge tube, and add 180 ul of Buffer NCL.Check ! If precipitated, Heat dissolve. (55°C)**

It is important to cut the tissue into small pieces to decrease lysis time.

If DNA is prepared from spleen tissue, no more than 10 mg should be used.

2. **Add 20 ul Proteinase K, mix by vortexing, and incubate at 56°C until the tissue is completely lysed.**

Note: Proteinase K has reduced activity in the presence of Buffer NCL. Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1.3 h. Lysis overnight is possible and does not influence the preparation. In order to ensure efficient lysis, a shaking water bath or a rocking platform should be used.

If not available, vortexing 2-3 times per hour during incubation is recommended.

3. **Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid. Continue with step 3a, or if RNA-free genomic DNA is required, continue with step 3b.**

Transcriptionally active tissues, such as liver and kidney, contain high levels of RNA which will copurify with genomic DNA. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

- 3a. **Add 200 ul Buffer NL to the sample, mix by pulse-vortexing for 15 s, and incubate at 70°C for 10 min. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.**

A white precipitate may form on addition of Buffer NL, in most cases it will dissolve during incubation at 70°C.

The precipitate does not interfere with the procedure, or with any subsequent application.

OR

- 3b. **First add 4 ul RNase A (100 mg/ml), mix by pulse-vortexing for 15 s, and incubate for 2 min at room temperature. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid before adding 200 ul Buffer NL to the sample. Mix again by pulse-vortexing for 15 s, and incubate at 70°C for 10 min.**

Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.

A white precipitate may form on addition of Buffer NL. In most cases it will dissolve during incubation at 70°C.

The precipitate does not interfere with the procedure or with any subsequent application.

4. **Add 400 ul ethanol (96-100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.**

A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the spin column. This precipitate does not interfere with the procedure or with any subsequent application.

5. **Carefully apply the mixture from step 4 (including the precipitate) to the spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the spin column in a clean 2 ml collection tube, and discard the tube containing the filtrate.**

It is essential to apply all of the precipitate to the spin column. If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all the solution has passed through.

6. **Carefully open the spin column and add 700 ul Wash C Solution without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min.**

Place the spin column in a clean 2 ml collection tube, and discard the tube containing the filtrate.

7. **Carefully open the spin column and add 700 ul Wash D Solution without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Finally centrifuge for 2 min at max. speed to remove residual wash solution.**
8. **Place the spin column in a clean 1.5 ml microcentrifuge tube, and discard the collection tube containing the filtrate. Carefully open the spin column and add 200 ul Elution Buffer or distilled water. Incubate at room temperature for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.**
9. **Repeat step 8.**
A 5 min incubation of the spin column loaded with Elution Buffer or water, before centrifugation, generally increases DNA yield.

Additional Protocols

Protocols for bacteria

These protocols have been used successfully for bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Bordetella pertussis* from nasopharyngeal swabs, *Borrelia burgdorferi* from cerebrospinal fluid, and *Legionella pneumophila* from broncho-alveolar lavage.

Please refer to For Gram-positive bacteria for other bacteria, especially other Gram-positive bacteria, which may be difficult to lyse.

For biological fluids

1. Pellet bacteria by centrifugation for 10 min at 5000 x g (7500 rpm).
2. Resuspend bacterial pellet in 180 ul Buffer NCL
3. Follow the Genomic Cell/Tissue Spin Mini Kit Protocol from step 2.

For eye, nasal, pharyngeal, or other swabs*

1. Collect samples and place in 2 ml PBS containing a common fungicide. Incubate for several hours at room temperature.
2. Follow Protocol A from step 1.

For bacterial cultures

a) Plate cultures

1. Remove bacteria from culture plate with an inoculation loop and suspend in 180 ul of Buffer NCL by vigorous stirring.
2. Follow the Genomic Cell/Tissue Spin Mini Kit Protocol from step 2.

b) Suspension cultures

1. Pipet 1 ml of bacterial culture into a 1.5 ml microcentrifuge tube, and centrifuge for 5 min at 5000 x g (7500 rpm).
2. Calculate the volume of the pellet or concentrate and add Buffer NCL to a total volume of 180 ul.
3. Follow the Genomic Cell/Tissue Spin Mini Kit Protocol from step 2.

Some bacteria (particularly Gram-positive bacteria) require pre-incubation with specific enzymes such as lysozyme or lysostaphin (e.g., staphylococci) to lyse the rigid multilayered cell wall. In these cases For Gram-positive bacteria should be used.

For Gram-positive bacteria

1. Pellet bacteria by centrifugation for 10 min at 5000 x g (7500 rpm).
2. Suspend bacterial pellet in 180 ul of the appropriate enzyme solution (20 mg/ml lysozyme or 200 ug/ml lysostaphin; 20 mM Tris·HCl, pH 8.0; 2 mM EDTA; 1.2% Triton®).
3. Incubate for at least 30 min at 37°C.
4. Add 20 ul Proteinase K and 200 ul Buffer NL. Mix by vortexing.
5. Incubate at 56°C for 30 min and then for a further 15 min at 95°C.
Note: Extended incubation at 95°C can lead to some DNA degradation.
6. Centrifuge for a few seconds.
7. Follow the Genomic Cell/Tissue Spin Mini Kit Protocol from step 4.

Protocol for Yeast

The procedure has been successfully used to isolate DNA from yeast. In this protocol, the cell wall of yeast cells is lysed enzymatically with lyticase. Spheroplasts are then collected by centrifugation.

Additional reagents required

When working with chemicals, always wear a suitable lab coat, disposable gloves, protective goggles.

- Sorbitol buffer: 1 M sorbitol
100 mM EDTA
14 mM β -mercaptoethanol

 - Lyticase (yeast-lysing enzyme)
1. **Harvest cells (max. 5×10^7) by centrifuging for 10 min at 5000 x g (7500 rpm). Discard supernatant.**
 2. **Resuspend the pellet in 600 μ l sorbitol buffer. Add 200 units lyticase and incubate at 30°C for 30 min.**
Note: Lysis time and yield will vary from sample to sample depending on the cell number and species processed.
 3. **Pellet the spheroplasts by centrifuging for 10 min at 300 x g.**
 4. **Resuspend the spheroplasts in 180 μ l Buffer NCL.**
 5. **Follow the Genomic Cell/Tissue Spin Mini Kit Protocol from step 2.**