# Genomic Blood Spin Kits

☐ **Genomic Blood Spin Mini Kit**

☐ **Genomic Blood Spin Midi Kit**

☐ **Genomic Blood Spin Maxi Kit**

NucleoGen
BIOTECHNOLOGY

# Contents

## Kit Contents

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| **Catalog No.** | **1515 / 1512** | | **6520 / 6550 / 6500** | | | **7506 / 7524 / 7550** | | |
| Number of preparations | 50 | 200 | 20 | 50 | 100 | 6 | 24 | 50 |
| Nucleogen Spin Columns | 50 | 200 | 20 | 50 | 100 | 6 | 24 | 50 |
| Collection tubes (2 ml) | 50 | 200 | 20 | 50 | 100 | 6 | 24 | 50 |
| Buffer NL* | + | + | + | + | + | + | + | + |
| Wash C Solution (concentrate)* | + | + | + | + | + | + | + | + |
| Wash D Solution (concentrate) | + | + | + | + | + | + | + | + |
| Elution Buffer | + | + | + | + | + | + | + | + |
| Proteinase K† | + | + | + | + | + | + | + | + |

* Buffer NL and Wash C Solution contain chaotropic salts which are irritants and not compatible with disinfecting agents containing bleach. Take appropriate laboratory safety measures and wear gloves when handling.
† Provided as a 20 mg/ml solution.

# Genomic Blood Spin Kits

## Principle

Genomic Blood Spin Mini Kits are designed for rapid purification of an average of 6 ug of total DNA (e.g., genomic, viral, mitochondrial) from 200 ul of whole human blood, and up to 50 ug of DNA from 200 ul of buffy coat, 5 x $10^6$ lymphocytes, or cultured cells that have a normal set of chromosomes.

Genomic Blood Spin Midi and Maxi Kits provide the fastest and easiest way to purify total DNA for reliable PCR and Southern blotting. Total DNA (e.g., genomic, viral, mitochondrial) can be purified from up to 0.3–2 ml and 3–10 ml, respectively, of whole blood, plasma, serum, buffy coat, bone marrow, other body fluids, and lymphocytes.

The procedure is suitable for use with whole blood treated with citrate, heparin, or EDTA; buffy coat; lymphocytes; plasma; serum; and body fluids. Samples may be either fresh or frozen. For larger volumes of whole blood or cultured cells we recommend using Nucleogen Genomic Blood Spin Midi and Maxi Kits.

**Amounts of starting material**

Small samples should be adjusted to 200 ul (Midi; 2 ml, Maxi; 10 ml) with PBS before loading, while for samples larger than 200 ul (Midi; 2 ml, Maxi; 10 ml), the amount of lysis buffer and other reagents added to the sample before loading must be increased proportionally. Application of the lysed sample to the Genomic Blood Spin Column will require more than one loading step if the initial sample volume is increased. The amounts of Wash C Solution and Wash D Solution used in the wash steps need not be increased.

**Sample volumes**

The Genomic Blood Spin Midi and Maxi procedures have been adapted for large sample volumes of 1–2 ml and 5–10 ml, respectively. The Midi procedure is optimized for use with 2 ml sample volumes, but samples as small as 300 ul can be prepared. In this case sample volumes should be adjusted to 1 ml with PBS. For samples >1 ml, the volumes of Buffer NL and ethanol should be adjusted proportionally, and the lysed sample should be loaded onto the Midi column in two steps, loading half the lysate in each step.

Samples with volumes less than 300 ul should be processed using the standard-sized Genomic Blood Spin columns.

If the initial sample volume exceeds 2 ml we recommend using Genomic Blood Spin Maxi columns.

The Maxi procedure is optimized for use with 10 ml sample volumes, but samples as small as 3 ml can be prepared. In this case sample volumes should be adjusted to 5 ml with PBS. For samples >5 ml, volumes of lysis Buffer NL and ethanol should be adjusted proportionally, and the lysed sample should be loaded onto the Genomic Spin Maxi column in two steps, loading half the lysate in each step.

**Cell number**

To achieve optimal binding conditions, it is important to avoid loading viscous lysates onto the Genomic Blood Spin columns. Lysate viscosity will increase proportionally with the number of cells present in the sample. The cell number should be determined before starting. No more than 2 x $10^7$ cells should be used with Genomic Blood Spin Midi columns. If your sample contains more than 2 x $10^7$ cells and up to 1 x $10^8$ cells, use the Genomic Blood Spin Maxi Kits to prepare genomic DNA. The Genomic Blood Spin Maxi Kit can be used to extract genomic DNA from blood containing as little as 2.5 x $10^5$ leukocytes per ml and up to 1 x $10^7$ cells per ml.

If you wish to prepare genomic DNA from blood containing more than 1 x $10^8$ cells, we suggest using doubled volumes of Buffer NL and ethanol, as this will reduce the viscosity of the lysate, and loading the lysate in repeated steps. Also, it is a good idea to increase the volume of Elution Buffer used for elution when preparing DNA from more than 1 x $10^8$ cells. Yields in excess of 1 mg have been achieved from 2.5 x $10^8$ cells although these yields are no longer quantitative since the amounts exceed the limits of linearity of the system.

In some cases it may be desired to process a large volume of blood in order to prepare DNA from a very small number of cells, e.g., to monitor the success of therapy after treatment.
Here, the Genomic Blood Spin Maxi Kits should be used to avoid repeated loading steps.
Typically, a sample containing 2.5 x $10^5$ cells per ml will yield approximately 16 ug of genomic DNA using the Genomic Blood Spin Maxi columns.

**Adsorption to the Nucleogen membrane**
The lysate buffering conditions are adjusted to allow optimal binding of the DNA to the membrane before the sample is loaded onto the Genomic Blood Spin column. DNA is adsorbed onto the membrane during a brief centrifugation step. Salt and pH conditions in the lysate ensure that protein and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not retained on the membrane. If the initial sample volume is larger than 200 ul, it will be necessary to load the lysate onto the Genomic Blood Spin Column in several steps. If larger sample volumes are required, we suggest using Genomic Spin Midi or Maxi Kits (Midi: 1–2 ml; Maxi: 5–10 ml starting material).

**Removal of residual contaminants**
DNA bound to the membrane is washed in two centrifugation steps.
The use of two different wash buffers, Wash C Solution and Wash D Solution, significantly improves the purity of the eluted DNA. Wash conditions ensure complete removal of any residual contaminants without affecting DNA binding.

**Elution of pure nucleic acids**
Purified DNA is eluted from the Genomic Blood Spin Column in a concentrated form in either Elution Buffer or Water. Elution Buffer should be equilibrated to room temperature (15–25°C) before it is applied to the column.
Yields will be increased if the Genomic Blood Spin Column is incubated with the elution buffer at room temperature for 5 minutes before centrifugation.

**Genomic Blood Spin Midi and Maxi Kit:** Two elution modes are possible: either elution with two separate volumes of elution buffer or elution with one volume of buffer and re-elution with the first eluate. Elution with two separate volumes increases DNA yield while re-elution increases DNA concentration.

With Maxi-columns, elution volumes larger than 1 ml increase DNA yield but reduce the concentration. Conversely, elution with volumes of less than 1 ml increases the final DNA concentration in the eluate significantly, but reduces overall yield. Similar results are achieved using Midi-columns.

If the total cell number exceeds 1 x $10^8$ we recommend increasing the volume of Elution Buffer up to 2 ml (Genomic Blood Spin Maxi Kit). Conversely, if the cell number is less than 2.5 x $10^5$, the volume of Elution Buffer should be reduced. We therefore suggest adjusting elution volumes according to the cell number and the researcher's individual requirements.

**Note: Do not reduce the elution volume below 100 ul when using Genomic Blood Spin Midi-columns or below 500 ul when using Genomic Blood Spin Maxi-columns.**

The eluted genomic DNA is up to 50 kb in length (predominantly 20–30 kb) and is suitable for direct use in PCR or Southern-blotting applications.
If the purified DNA is to be stored, elution in Elution Buffer (10 mM Tris·Cl; pH 8.5) and storage at –20°C is recommended. DNA stored in water is subject to degradation by acid hydrolysis.

**Elution mode for maximum yield or concentration (Mini)**
The yield of genomic DNA depends on the sample type and the number of cells in the sample. Typically, a 200 ul sample of whole blood from a healthy individual will yield 3–12 ug of DNA. (If higher yields are required, use Genomic Blood Spin Midi or Maxi Kits with up to 2 ml or up to 10 ml blood, respectively.) For most whole blood samples, a single elution with 200 ul Elution Buffer is sufficient. Samples with elevated white blood cell (WBC) counts, ranging from 1 x $10^7$ to 1.5 x $10^7$ cells/ml, will yield between 13 and 20 ug of DNA. If such a sample is loaded onto the column, approximately 80% of the DNA will elute in the first 200 ul, and up to 20% more in the next 200 ul. In samples with WBC counts exceeding 1.5 x $10^7$ cells/ml, up to 60% of the DNA will elute in the first 200 ul and up

to 70% of the remaining material in each subsequent 200 ul. Elution into a fresh tube is recommended to prevent dilution of the first eluate. More than 200 ul should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will contact the eluate, leading to possible aerosol formation of samples during centrifugation. Eluting in 4 x 100 ul instead of 2 x 200 ul does not increase elution efficiency. In all cases, a single elution with 200 ul of Elution Buffer will provide sufficient DNA to perform multiple amplification reactions.

For some downstream applications, concentrated DNA may be required. Elution with volumes of less than 200 ul increases the final DNA concentration in the eluate significantly but slightly reduces overall DNA yield.

For samples containing <3 ug of DNA, eluting the DNA in 100 ul is recommended. For samples containing less than 1 ug of DNA, only one elution in 50 ul Elution Buffer or Water is recommended.

**Growth area and number of HeLa cells in various culture dishes**

| Cell culture vessel | Growth area* ($cm^2$) | Number of cells† |
|---|---|---|
| **Multiwell plates** | | |
| 96-well | 0.32-0.6 | 4-5 x $10^4$ |
| 48-well | 1 | 1 x $10^5$ |
| 24-well | 2 | 2.5 x $10^5$ |
| 12-well | 4 | 5 x $10^5$ |
| 6-well | 9.5 | 1 x $10^6$ |
| **Dishes** | | |
| Ø 35 mm | 8 | 1 x $10^6$ |
| Ø 60 mm | 21 | 2.5 x $10^6$ |
| Ø 100 mm | 56 | 7 x $10^6$ |
| Ø 145-150 mm | 145 | 2 x $10^7$ |
| **Flasks** | | |
| 40-50 ml | 25 | 3 x $10^6$ |
| 250-300 ml | 75 | 1 x $10^7$ |
| 650-750 ml | 162-175 | 2 x $10^7$ |

* Per well, if multiwell plate are used; varies slightly depending on the supplier.

† Cell numbers given are for HeLa cells (approximate length = 15 um) assuming confluent growth. Cell numbers vary since animal cells can vary in length from 10-100 um.

# Important Notes

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

**Before equipment**

- **Buffer NL:** 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 Soluiton**, 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.
- Prepare a 70°C water bath for use in the Midi and Maxi 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.
- Do not use a fixed-angle rotor (Midi and Maxi ).

**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 Blood Spin Mini Kit Protocol
## Using a Microcentrifuge

**Please read "Important Notes" on pages 6 before starting.**

1. **Pipet 20 ul Proteinase K into the bottom of a 1.5 ml microcentrifuge tube.**

2. **Add 200 ul sample to the microcentrifuge tube. Use up to 200 ul whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x $10^6$ lymphocytes in 200 ul PBS.**
   Mini-columns copurify RNA and DNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 ul of an RNase A stock solution (100 mg/ml) should be added to the sample before addition of Buffer NL.

3. **Add 200 ul Buffer NL to the sample. Mix by pulse-vortexing for 15 sec.**
   If the sample volume is larger than 200 ul, increase the amount of Proteinase K and Buffer NL proportionally; e.g., a 400 ul sample will require 40 ul Proteinase K and 400 ul Buffer NL.
   **Note:** Do not add Proteinase K directly to Buffer NL.

4. **Incubate at 56°C for 10 min.**
   Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

5. **Add 400 ul ethanol (96-100%) to the sample, and mix again by pulse-vortexing for 15 s.** After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
   If the sample volume is greater than 400 ul, increase the amount of alcohol proportionally; e.g., a 400 ul sample will require 800 ul of alcohol.

6. **Carefully apply the mixture from step 5 to the Mini-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 Mini-column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.
   If the lysate has not completely passed through the Mini-column after centrifugation, centrifuge again at higher speed until the Mini-column is empty.
   **Note:** When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.

7. **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 (provided), and discard the collection tube containing the filtrate.
   It is not necessary to increase the volume of Wash C Solution if the original sample volume is larger than 200 ul.

8. **Carefully open the spin column and add 700 ul Wash D Solution without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.** Continue directly with step 9, or to eliminate any chance of possible Wash D Solution carryover, perform step 8a, and then continue with step 9.

   8a. **(Optional): Place the spin column in a new 2 ml collection tube (not provided) and discard the collection tube with the filtrate. Centrifuge at 20,000 x g (14,000 rpm) for 1 min.**

9. **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 (15-25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min**.
   Incubating the spin column loaded with Elution Buffer or water for 5 min at room temperature before centrifugation generally increases DNA yield.
   A second elution step with a further 200 ul Elution Buffer will increase yields by up to 15%.
   Elution with volumes of less than 200 ul increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. For samples containing less than 1 ug of DNA, elution in 50 ul Elution Buffer or water is recommended. Eluting with 2 x 100 ul instead of 1 x 200 ul does not increase elution efficiency.

# Genomic Blood Spin Mini Kit Protocol
## Using a Vacuum Manifold

**Please read "Important Notes" on pages 6 before starting.**

1. **Pipet 20 ul Proteinase K into the bottom of a 1.5 ml microcentrifuge tube.**
2. **Add 200 ul sample to the microcentrifuge tube. Use up to 200 ul whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x $10^6$ lymphocytes in 200 ul PBS.**
   Spin columns copurify RNA and DNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 ul of an RNase A stock solution (100 mg/ml) should be added to the sample before addition of Buffer NL.
3. **Add 200 ul Buffer NL to the sample. Mix by pulse-vortexing for 15 sec.**
   If the sample volume is larger than 200 ul, increase the amount of Proteinase K and Buffer NL proportionally; e.g., a 400 ul sample will require 40 ul Proteinase K and 400 ul Buffer NL.
   **Note:** Do not add Proteinase K directly to Buffer NL.
4. **Incubate at 56°C for 10 min.**
   Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
5. **Add 400 ul ethanol (96-100%) to the sample, and mix again by pulse-vortexing for 15 s.** After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
   If the sample volume is greater than 400 ul, increase the amount of alcohol proportionally; e.g., a 400 ul sample will require 800 ul of alcohol.
   **Prepare the vacuum manifold and Mini-columns:**
   - Insert each Mini-column into a luer connector on the luer adapter(s) in the vacuum manifold.
6. **Carefully apply the mixture from step 5 to the Mini Column.**
7. **Switch on vacuum source to draw the solution through the Mini-columns, and then switch off vacuum source.**
8. **Wash the Mini-column by adding 700 ul Wash C Solution. Switch on vacuum source to draw the wash C solution through the Mini-column, and then switch off vacuum source.**
9. **Wash the Mini-column by adding 700 ul Wash D Solution. Switch on vacuum source to draw the wash D solution through the Mini-column, and then switch off vacuum source.**
10. **Transfer the Mini-column to a microcentrifuge tube. Centrifuge for 1 min.**
    **IMPORTANT:** This extra spin is necessary to remove residual Wash D Solution. Residual ethanol from Wash D Solution may inhibit subsequent enzymatic reactions.
11. **Place the spin column in a clean 1.5 ml microcentrifuge tube, and discard the collection tube containing the filtrate. Carefully open the Mini-column and add 200 ul Elution Buffer or distilled water. Incubate at room temperature (15-25°C) for 1 min, and then centrifuge at 6,000 x g (8,000 rpm) for 1 min**.
    Incubating the spin column loaded with Elution Buffer or water for 5 min at room temperature before centrifugation generally increases DNA yield.
    A second elution step with a further 200 ul Elution Buffer will increase yields by up to 15%.
    Elution with volumes of less than 200 ul increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. For samples containing less than 1 ug of DNA, elution in 50 ul Elution Buffer or water is recommended. Eluting with 2 x 100 ul instead of 1 x 200 ul does not increase elution efficiency.

# Genomic Blood Spin Midi Kit Protocol

For isolation of genomic DNA from **2 ml** (1 ml) of whole blood.

## Using a Centrifuge

**Please read "Important Notes" on pages 6 before starting.**

1. **Pipet 200 ul Proteinase K into the bottom of a 15 ml centrifuge tube.**
   *If the sample volume is less than 2 ml, reduce the amount of Protease proportionally, e.g., use 100 ul Protease for 1 ml blood.*

2. **Add 2 ml blood and mix briefly.**
   *If sample volume is less than 1 ml, add the appropriate volume of PBS.*

3. **Add 2.4 ml Buffer NL and mix thoroughly by vortexing at least 3 times, for 5 s each time.**
   *If the initial sample volume was less than 2 ml, reduce the amount of Buffer NL proportionally, e.g., use 1.2 ml Buffer NL for 1 ml blood.*
   **Note:** Do not add Protease directly to Buffer NL.

4. **Incubate at 70°C for 10 min.**

5. **Add 3.6 ml ethanol (96–100%) to the sample and mix again by vortexing.**
   *If the initial sample volume was less than 2 ml, reduce the amount of ethanol proportionally, e.g., use 2 ml ethanol for 1 ml blood.*

6. **Carefully transfer half of the solution (4.1 ml) from step 4 onto the Midi column placed in a 15 ml centrifugation tube. Avoid spilling and do not moisten the rim of the Midi column. Close the cap and centrifuge at 1850 x g (3000 rpm) for 3 min.**
   *If the initial sample volume was 1 ml, apply all the lysate to the Midi column. Close the cap and centrifuge as above. Proceed directly to step 8.*
   If the solution has not completely passed through the membrane, centrifuge again at a slightly higher speed.

7. **Remove the Midi column, discard the filtrate, and place the Midi column back into the 15 ml centrifugation tube. Load the remainder of the solution from step 5 onto the Midi column. Close the cap and recentrifuge at 1850 x *g* (3000 rpm) for 3 min.**
   **Note:** Wipe off any spillage from the thread of the 15 ml centrifugation tube before re-inserting the Midi column. If the solution has not completely passed through the membrane, centrifuge again at a slightly higher speed.

8. **Remove the Midi column, discard the filtrate, and place the Midi column back into the 15 ml centrifugation tube.**
   **Note:** Wipe off any spillage from the thread of the 15 ml centrifugation tube before re-inserting the Midi column.

9. **Carefully, without moistening the rim, add 2.5 ml Wash C Solution to the Midi column. Close the cap and centrifuge at 4500 x g (5000 rpm) for 1 min**.
   **Note:** Do not discard the flow-through at this stage and continue directly with step 10.

10. **Carefully, without moistening the rim, add 2.5 ml of Wash D Solution to the Midi column. Close the cap and centrifuge at 4500 x g (5000 rpm) for 15 min**.
    **Note:** The increased centrifugation time should remove all traces of Wash D Solution from the Midi column before elution. If the centrifugal force is below 4000 x g, incubating the Midi column for 10 min at 70°C in an incubator to evaporate residual ethanol is recommended.

11. **Place the Midi column in a clean 15 ml centrifugation tube, and discard the collection tube containing the filtrate.**
    **Note:** Wipe any spillage off the Midi column before insertion into the clean 15 ml centrifugation tube.

12. **Add 300 ul of Elution Buffer or distilled water, equilibrated to room temperature (15–25°C). Pipet directly onto the membrane of the Midi column and close the cap. Incubate at room temperature for 5 min and centrifuge at 4500 x g (5000 rpm) for 5 min.**
*If the initial sample volume was 1 ml, pipet 200 ul of Elution Buffer or distilled water, equilibrated to room temperature, onto the membrane of the Midi column and close the cap. Incubate at room temperature for 5 min and centrifuge at 4500 x g (5000 rpm) for 5 min.*

13. **To obtain highly concentrated DNA proceed to step 13a. To obtain maximum DNA yield proceed to step 13b.**
**Note:** Highly concentrated DNA may be desirable for applications such as restriction digestion and Southern blotting. For other applications, increasing the total DNA yield may be preferable.

    13a. **maximum DNA concentration: Reload the 300 ul of eluate containing the DNA onto the membrane of the Midi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x g (5000 rpm) for 5 min.**
    *If the initial sample volume was 1 ml, reload the 200 ul of eluate containing the DNA onto the membrane of the Midi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x g (5000 rpm) for 5 min.*
    **Note:** Less than 300 ul will be eluted from the column, but this has no influence on DNA yield.

    13b. **For maximum DNA yield: Pipet 300 ul of fresh Elution Buffer or distilled water, equilibrated to room temperature, onto the membrane of the Midi column. Incubate at room temperature for 5 min. Close the cap and centrifuge at 4500 x g (5000 rpm) for 5 min.**
    *If the initial sample volume was 1 ml, pipet 200 ul of fresh Elution Buffer or distilled water, equilibrated to room temperature, onto the membrane of the Midi column.*
    *Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x g (5000 rpm) for 5 min.*
    **Note:** Less than 300 ul will be eluted from the column, but this has no influence on DNA yield.

# Genomic Blood Spin Midi Kit Protocol
## Using a Vacuum Manifold

**Please read "Important Notes" on pages 6 before starting.**

1. **Pipet 200 ul Proteinase K into the bottom of a 15 ml centrifuge tube.**
   *If the sample volume is less than 2 ml, reduce the amount of Protease proportionally, e.g., use 100 ul Protease for 1 ml blood.*

2. **Add 2 ml blood and mix briefly.**
   *If sample volume is less than 1 ml, add the appropriate volume of PBS.*

3. **Add 2.4 ml Buffer NL and mix thoroughly by vortexing at least 3 times, for 5 s each time.**
   *If the initial sample volume was less than 2 ml, reduce the amount of Buffer NL proportionally, e.g., use 1.2 ml Buffer NL for 1 ml blood.*
   **Note:** Do not add Protease directly to Buffer NL.

4. **Incubate at 70°C for 10 min.**

5. **Add 3.6 ml ethanol (96–100%) to the sample and mix again by vortexing.**
   *If the initial sample volume was less than 2 ml, reduce the amount of ethanol proportionally, e.g., use 2 ml ethanol for 1 ml blood.*
   **Prepare the vacuum manifold and Midi-columns:**
   - Insert each Midi-column into a luer connector on the luer adapter(s) in the vacuum manifold.

6. **Carefully apply the mixture from step 5 to the Midi-column.**

7. **Switch on vacuum source to draw the solution through the Midi-columns, and then switch off vacuum source.**

8. **Wash the Midi-column by adding 2.5 ml Wash C Solution. Switch on vacuum source to draw the wash C solution through the Mini-column, and then switch off vacuum source.**

9. **Wash the Midi-column by adding 2.5 ml Wash D Solution. Switch on vacuum source to draw the wash D solution through the Mini-column, and then switch off vacuum source.**

10. **Transfer the Midi-column to a centrifuge tube. Centrifuge for 1 min.**
    **IMPORTANT:** This extra spin is necessary to remove residual Wash D Solution. Residual ethanol from Wash D Solution may inhibit subsequent enzymatic reactions.

11. **Place the Midi-column in a clean 15 ml centrifuge tube, and discard the collection tube containing the filtrate.**
    **Note:** Wipe any spillage off the Midi column before insertion into the clean 15 ml centrifugation tube.

12. **Add 300 ul of Elution Buffer or distilled water, equilibrated to room temperature (15–25°C). Pipet directly onto the membrane of the Midi column and close the cap. Incubate at room temperature for 5 min and centrifuge at 4500 x g (5000 rpm) for 5 min.**
    *If the initial sample volume was 1 ml, pipet 200 ul of Elution Buffer or distilled water, equilibrated to room temperature, onto the membrane of the Midi column and close the cap. Incubate at room temperature for 5 min and centrifuge at 4500 x g (5000 rpm) for 5 min.*

13. **To obtain highly concentrated DNA proceed to step 13a. To obtain maximum DNA yield proceed to step 13b.**
    **Note:** Highly concentrated DNA may be desirable for applications such as restriction digestion and Southern blotting. For other applications, increasing the total DNA yield may be preferable.

13a. **maximum DNA concentration: Reload the 300 ul of eluate containing the DNA onto the membrane of the Midi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x g (5000 rpm) for 5 min.**

*If the initial sample volume was 1 ml, reload the 200 ul of eluate containing the DNA onto the membrane of the Midi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x g (5000 rpm) for 5 min.*

**Note:** Less than 300 ul will be eluted from the column, but this has no influence on DNA yield.

13b. **For maximum DNA yield: Pipet 300 ul of fresh Elution Buffer or distilled water, equilibrated to room temperature, onto the membrane of the Midi column. Incubate at room temperature for 5 min. Close the cap and centrifuge at 4500 x g (5000 rpm) for 5 min.**

*If the initial sample volume was 1 ml, pipet 200 ul of fresh Elution Buffer or distilled water, equilibrated to room temperature, onto the membrane of the Midi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x g (5000 rpm) for 5 min.*

**Note:** Less than 300 ul will be eluted from the column, but this has no influence on DNA yield.

# Genomic Blood Spin Maxi Kit Protocol

For isolation of genomic DNA from **10 ml** (5 ml) of whole blood.

## Using a Centrifuge

**Please read "Important Notes" on pages 6 before starting.**

1. **Pipet 500 ul Proteinase K into the bottom of a 50 ml centrifuge tube.**
   *If the sample volume is less than 10 ml, use the same amount of Proteinase K.*
2. **Add 10 ml blood and mix briefly.**
   *If sample volume is less than 5 ml, add the appropriate volume of PBS.*
3. **Add 12 ml Buffer NL and mix thoroughly by vortexing at least 3 times, for 5 s each time.**
   *If the initial sample volume was less than 10 ml, reduce the amount of Buffer NL proportionally, e.g., use 6 ml Buffer NL for 5 ml blood.*
   **Note:** Do not add Protease directly to Buffer NL.
4. **Incubate at 70°C for 10 min.**
5. **Add 20 ml of ethanol (96–100%) to the sample and mix again by vortexing.**
   *If the initial sample volume was less than 10 ml, reduce the amount of ethanol proportionally, e.g., use 10 ml ethanol for 5 ml blood.*
6. **Carefully apply half of the solution (21.25 ml) from step 5 onto the Maxi column placed in a 50 ml centrifugation tube. Avoid spilling and do not moisten the rim of the Maxi column. Close the cap and centrifuge at 1850 x g (3000 rpm) for 3 min.**
   *If the initial sample volume was 5 ml, apply all the lysate to the Maxi column. Close the cap and centrifuge as above. Proceed directly to step 8.*
   If the solution has not completely passed through the membrane, centrifuge again at a slightly higher speed.
7. **Remove the Maxi column, discard the filtrate, and place the Maxi column back into the 50 ml centrifugation tube. Load the remainder of the solution from step 5 onto the Maxi column. Close the cap and recentrifuge at 1850 x g (3000 rpm) for 3 min.**
   **Note:** Wipe off any spillage from the thread of the 50 ml centrifugation tube before re-inserting the Maxi column.
   If the solution has not completely passed through the membrane centrifuge again at a slightly higher speed.
8. **Remove the Maxi column, discard the filtrate, and place the Maxi column back into the 50 ml centrifugation tube.**
   **Note:** Wipe off any spillage from the thread of the 50 ml centrifugation tube before re-inserting the Maxi column.
9. **Carefully, without moistening the rim, add 6 ml Wash C Solution to the Maxi column. Close the cap and centrifuge at 4500 x g (5000 rpm) for 1 min**.
   **Note:** Do not discard the flow-through at this stage and continue directly with step 10.
10. **Carefully, without moistening the rim, add 6 ml of Wash D Solution to the Maxi column. Close the cap and centrifuge at 4500 x g (5000 rpm) for 15 min**.
    **Note:** The increased centrifugation time should be sufficient to remove all traces of Wash D Solution from the Maxi column before elution. If the centrifugal force is below 4000 x g, incubating the Maxi column for 10 min at 70°C in an incubator to evaporate residual ethanol is recommended.
11. **Discard the 50 ml centrifugation tube containing the filtrate, and place the Maxi column in a clean 50 ml centrifugation tube.**
    **Note:** Wipe any spillage off the Maxi column before insertion into the clean 50 ml centrifugation tube.

12. **Add 1 ml of Elution Buffer, or distilled water, equilibrated to room temperature (15–25°C). Pipet directly onto the membrane of the Maxi column and close the cap. Incubate at room temperature for 5 min and centrifuge at 4500 x g (5000 rpm) for 5 min.**
*If the initial sample volume was 5 ml, pipet 600 ul of Elution Buffer or distilled water, equilibrated to room temperature, onto the membrane of the Maxi column and close the cap. Incubate at room temperature for 5 min and centrifuge at 4500 x g (5000 rpm) for 5 min.*

13. **To obtain highly concentrated DNA proceed to step 13a. To obtain maximum DNA yield proceed to step 13b.**
**Note:** Highly concentrated DNA may be desirable for applications such as restriction digestion and Southern blotting. For other applications, increasing the total DNA yield may be preferable .

    13a. **For maximum concentration: Reload the 1 ml of eluate containing the DNA onto the membrane of the Maxi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x g (5000 rpm) for 5 min.**
    *If the initial sample volume was 5 ml, reload the 600 µl of eluate containing the DNA onto the membrane of the Maxi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x* g *(5000 rpm) for 5 min.*
    **Note:** Less than 1 ml will be eluted from the column, but this has no effect on DNA yield.

    13b. **For maximum yield: Pipet 1 ml of fresh Elution Buffer or distilled water, equilibrated to room temperature, onto the membrane of the Maxi column. Incubate at room temperature for 5 min and centrifuge at 4500 x *g* (5000 rpm) for 5 min.**
    *If the initial sample volume was 5 ml, pipet 600 ul of fresh Elution Buffer or distilled water, equilibrated to room temperature, onto the membrane of the Maxi column.*
    *Incubate at room temperature for 5 min and centrifuge at 4500 x g (5000 rpm) for 5 min.*
    **Note:** Less than 1 ml will be eluted from the column, but this has no effect on DNA yield.

# Genomic Blood Spin Maxi Kit Protocol
## Using a Vacuum Manifold

**Please read "Important Notes" on pages 6 before starting.**

1. **Pipet 500 ul Proteinase K into the bottom of a 50 ml centrifuge tube.**
   *If the sample volume is less than 10 ml, use the same amount of Proteinase K.*
2. **Add 10 ml blood and mix briefly.**
   *If sample volume is less than 5 ml, add the appropriate volume of PBS.*
3. **Add 12 ml Buffer NL and mix thoroughly by vortexing at least 3 times, for 5 s each time.**
   *If the initial sample volume was less than 10 ml, reduce the amount of Buffer NL proportionally, e.g., use 6 ml Buffer NL for 5 ml blood.*
   **Note:** Do not add Protease directly to Buffer NL.
4. **Incubate at 70°C for 10 min.**
5. **Add 20 ml ethanol (96–100%) to the sample and mix again by vortexing.**
   *If the initial sample volume was less than 10 ml, reduce the amount of ethanol proportionally, e.g., use 10 ml ethanol for 5 ml blood.*
   **Prepare the vacuum manifold and Maxi-columns:**
   - Insert each Maxi-column into a luer connector on the luer adapter(s) in the vacuum manifold.
6. **Carefully apply the mixture from step 5 to the Maxi-column.**
7. **Switch on vacuum source to draw the solution through the Maxi-columns, and then switch off vacuum source.**
8. **Wash the Maxi-column by adding 6 ml Wash C Solution. Switch on vacuum source to draw the wash C solution through the Mini-column, and then switch off vacuum source.**
9. **Wash the Midi-column by adding 6 ml Wash D Solution. Switch on vacuum source to draw the wash D solution through the Mini-column, and then switch off vacuum source.**
10. **Transfer the Midi-column to a centrifuge tube. Centrifuge for 1 min.**
    **IMPORTANT:** This extra spin is necessary to remove residual Wash D Solution. Residual ethanol from Wash D Solution may inhibit subsequent enzymatic reactions.
11. **Place the Midi-column in a clean 50 ml centrifuge tube, and discard the collection tube containing the filtrate.**
    **Note:** Wipe any spillage off the Midi column before insertion into the clean 15 ml centrifugation tube.
12. **Add 1 ml of Elution Buffer, or distilled water, equilibrated to room temperature (15–25°C). Pipet directly onto the membrane of the Maxi column and close the cap. Incubate at room temperature for 5 min and centrifuge at 4500 x g (5000 rpm) for 5 min.**
    *If the initial sample volume was 5 ml, pipet 600 ul of Elution Buffer or distilled water, equilibrated to room temperature, onto the membrane of the Maxi column and close the cap. Incubate at room temperature for 5 min and centrifuge at 4500 x g (5000 rpm) for 5 min.*
13. **To obtain highly concentrated DNA proceed to step 13a. To obtain maximum DNA yield proceed to step 13b.**
    **Note:** Highly concentrated DNA may be desirable for applications such as restriction digestion and Southern blotting. For other applications, increasing the total DNA yield may be preferable .

    13a. **For maximum concentration: Reload the 1 ml of eluate containing the DNA onto the membrane of the Maxi column. Close the cap and incubate at room temperature for**

**5 min. Centrifuge at 4500 x g (5000 rpm) for 5 min.**
*If the initial sample volume was 5 ml, reload the 600 µl of eluate containing the DNA onto the membrane of the Maxi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x* g *(5000 rpm) for 5 min.*
**Note:** Less than 1 ml will be eluted from the column, but this has no effect on DNA yield.

13b. **For maximum yield: Pipet 1 ml of fresh Elution Buffer or distilled water, equilibrated to room temperature, onto the membrane of the Maxi column. Incubate at room temperature for 5 min and centrifuge at 4500 x *g* (5000 rpm) for 5 min.**
*If the initial sample volume was 5 ml, pipet 600 ul of fresh Elution Buffer or distilled water, equilibrated to room temperature, onto the membrane of the Maxi column. Incubate at room temperature for 5 min and centrifuge at 4500 x g (5000 rpm) for 5 min.*
**Note:** Less than 1 ml will be eluted from the column, but this has no effect on DNA yield.

## Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise.

| | Comments and suggestions |
|---|---|
| **Colored residues remain on the Genomic Spin Column after washing** | |
| a) Inefficient cell lysis due to insufficient mixing of the sample with Buffer NL | Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer NL immediately and thoroughly by pulse-vortexing. |
| a) Inefficient cell lysis due to decreased protease activity | Repeat the DNA purification procedure with a new sample and with freshly prepared Proteinase K stock solution. Be sure to store the stock solution at 2–8°C immediately after use. Ensure that Proteinase K is not added directly to Buffer NL. |
| b) No alcohol added to the lysate before loading onto the spin column | Repeat the purification procedure with a new sample. |
| c) Wash C or D Solution prepared incorrectly | Ensure that Wash C and D Solution concentrates were diluted with the correct volumes of pure ethanol. Repeat the purification procedure with a new sample. |
| **Little or no DNA in the eluate** | |
| a) Low concentration of cells in the sample | Mini: Concentrate a larger volume of a new cell-free sample to 200 ul. Repeat the DNA purification procedure, adding 5–10 ug of carrier DNA to each lysate if the sample has a low DNA content. If whole blood was used, prepare buffy coat. Midi/Maxi: Increase the sample volumes and load the Midi/Maxi column several times. Reduce volume of Elution Buffer used for elution. Repeat the DNA purification procedure with a new sample. |
| b) Inefficient cell lysis due to insufficient mixing with Buffer NL | Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer NL immediately and thoroughly by pulse-vortexing. |
| a) Inefficient cell lysis due to decreased protease activity | Repeat the DNA purification procedure with a new sample and with freshly prepared Proteinase K stock solution. Be sure to store the stock solution at 2–8°C immediately after use. Ensure that Proteinase K is not added directly to Buffer NL. |
| b) Inefficient cell lysis or protein degradation in Buffer NL or NCL due to insufficient incubation time | Repeat the procedure with a new sample. Ensure that the tissue sample is cut into small pieces and extend the incubation time. Ensure that no residual particulates are visible (bones or hair will not be lysed at all). |
| c) No alchol added to the lysate before loading onto the spin column | Repeat the purification procedure with a new sample. |
| d) Isopropanol used instead of ethanol with samples other than blood or plasma | We strictly recommend the use of ethanol with all samples other than blood or plasma (serum). The use of isopropanol results in reduced yields with all other samples. |
| h) Spin column not incubated at room temperature (15–25°C) for 1 min (Midi/Maxi, 5 min) | After addition of Elution Buffer or water, the Genomic spin column should be incubated at room temperature for at least 1 min (Midi/Maxi, 5 min). |

| | Comments and suggestions |
|---|---|
| i) DNA not eluted efficiently | To increase elution efficiency, pipet Elution Buffer or water onto the spin column and incubate the column for 5 min at room temperature before centrifugation. |
| j) pH of water incorrect (acidic) | Low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use Elution Buffer for elution. |
| k) Wash C or D Solution prepared incorrectly | Check that Wash C and D Solution concentrates were diluted with the correct volumes of pure ethanol. Repeat the purification procedure with a new sample. |
| l) Wash C or D Solution prepared with 70% ethanol | Check that Wash C and D Solution concentrates were diluted with 96–100% ethanol. Repeat the purification procedure with a new sample. |
| m) Wash C or D Solution used in the wrong order | Ensure that Wash C and D Solution are used in the correct order in the protocol. Repeat the purification procedure with a new sample. |
| n) Inefficient binding due to high viscosity of lysate | Repeat the DNA purification procedure with new samples. Use double the volume of Buffer NL. Mix the sample and Buffer NL immediately and thoroughly. After incubation, add double the amount of ethanol and mix thoroughly. Load sample several times onto the same Midi/Maxi column. |
| **Low concentration of DNA in the eluate** | |
| a) DNA eluted with 300 ul (Midi) or 1 ml (Maxi) of Elution Buffer or water | Elution with volumes of less than 300 ul (Midi), or 1 ml (Maxi) increases the final DNA concentration in the eluate, but slightly reduces overall DNA yield.<br>For samples containing less than 4 ug (Midi) or 8 ug (Maxi) of DNA, re-elution in 100 ul (Midi) or 400 ul (Maxi) of Elution Buffer or distilled water is recommended. |
| ***A*260/*A*280 ratio for purified nucleic acids is low** | |
| a) Inefficient cell lysis due to insufficient mixing with Buffer NL | Repeat the procedure with a new sample. Be sure to mix the sample and Buffer NL immediately and thoroughly by pulse-vortexing. |
| b) Inefficient cell lysis due to decreased proteinase K activity | Repeat the DNA purification procedure with a new sample and with freshly prepared Proteinase K stock solution.<br>Be sure to store the stock solution at 2–8°C immediately after use. Ensure that Proteinase K is not added directly to Buffer NL. |
| c) Inefficient cell lysis or protein degradation in Buffer NL or NCL due to insufficient incubation time | Repeat the procedure with a new sample. Extend the incubation time. Take care that no residual particulates are visible (bones or hair will not be lysed at all). |
| d) No alcohol added to the lysate before loading onto the spin column | Repeat the purification procedure with a new sample. |
| e) Low percentage alcohol used instead of 100% | Repeat the purification procedure with a new sample. |
| f) Wash C or D Solution prepared incorrectly | Check that Wash C and D Solution concentrates were diluted with the correct volumes of pure ethanol. Repeat the purification procedure with a new sample. |
| g) Wash C or D Solution prepared with 70% ethanol | Check that Wash C and D Solution concentrates were diluted with 96–100% ethanol. Repeat the purification procedure with a new sample. |

| | Comments and suggestions |
|---|---|
| h) Wash C and D Solution used in the wrong order | Ensure that Wash C and D Solution are used in the correct order in the protocol. Repeat the purification procedure with a new sample. |
| ***A*260/*A*280 ratio for purified nucleic acids is high** | |
| a) High level of residual RNA | In future DNA preparations, use the optional RNase step included in the protocols. |
| b) Buffer NL added to the sample before addition of RNase A | Always add RNase A first and vortex when using the optional RNase A step. |
| **DNA does not perform well in subsequent enzymatic reactions** | |
| a) Not enough DNA in sample | Check "Little or no DNA in the eluate" of this troubleshooting guide for possible reasons. Increase the amount of eluate added to the reaction, if possible. If necessary, vacuum-concentrate the DNA, or increase the amount of sample used and repeat the purification procedure. If the amount of purified DNA is still expected to be low, reduce the elution volume. Lowering the elution volume will slightly reduce overall yield, but will result in a higher concentration of nucleic acids in the eluate. DNA remaining on the Midi/Maxi column can be recovered in a subsequent elution step by applying the same eluate, heated to 70°C, to the column. |
| b) Inhibitory substances in preparation | Check "*A*260/*A*280 ratio for purified nucleic acids is low" of this troubleshooting guide for possible reasons. |
| c) Residual Wash D Solution In the eluate | Use optional drying step in the relevant protocol. Ensure that the spin column does not come into contact with the filtrate prior to elution. |
| d) Wash C and D Solution used in the wrong order | Ensure that Wash C and D Solution are used in the correct order in the protocol. Repeat the purification procedure with a new sample. |
| d) High level of residual RNA | In future DNA preparations, use the optional RNase step included in the protocols. |
| e) Reduced sensitivity of amplification reaction | Adjust the volume of eluate added as template in the amplification reaction. |
| f)Amplification reaction setup has been modified | Reoptimize the amplification system by adjusting the volume of eluate added. |
| **White precipitate in Buffer NCL or Buffer NL** | |
| a) White precipitate may form after storage at low temperature or prolonged storage | Any precipitate in Buffer NL must be dissolved by incubation of the buffer at 56°C. The precipitate has no effect on function. Dissolving the precipitate at high temperature will not compromise yield or quality of the purified nucleic acids. |
| **White precipitate in steps 3 or 4 of the tissue protocol** | |
| a) White precipitate may form on addition of Buffer NL in step 3 or Ethanol in step 4 | In most cases the precipitate formed in step 3 will dissolve during incubation at 70°C. The precipitates do not interfere with the Genomic Spin procedure, or with any subsequent application. |

# Additional Protocols

## Protocol for cultured cells

1. **Harvest cells: maximum 5 x $10^6$ cells, with a normal set of chromosomes**
   a) Cells grown in suspension
   Centrifuge the appropriate number of cells for 5 min at 300 x g in a 1.5 ml microcentrifuge tube. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 2.

   b) Cells grown in monolayer
   1) To trypsinize cells: Aspirate the medium and wash cells with PBS. Aspirate the PBS and add trypsin solution. After cells have become detached from the dish or flask, collect the cells in medium, and transfer the appropriate number of cells to a 1.5 ml microcentrifuge tube. Centrifuge for 5 min at 300 x g. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 2.

   2) Using a cell scraper, detach cells from the the dish or flask. Transfer the appropriate number of cells to a 1.5 ml microcentifuge tube and centrifuge for 5 min at 300 x g. Remove the supernatant completely and discard without disturbing the cell pellet. Continue with step 2.

2. **Resuspend cell pellet in PBS to a final volume of 200 ul.**
3. **Add 20 ul Proteinase K.**
4. **Continue with step 3 of the Genomic Blood Spin Mini Kit Protocol (page 7).**

Genomic Blood Spin Kits

## Protocol for Buccal Swab
### Using a Microcentrifuge

**Please read "Important Notes" on pages 6 before starting.**

**Important points before starting:**

- Due to the increased volume of Buffer NL that is required for the buccal swab protocol, fewer preparations can be performed.
- This protocol is recommended for the following swab types: C.E.P. (Omni Swabs from Whatman Bioscience), cotton, and DACRON® (Daigger, Puritan® applicators with plastic stick and cotton or DACRON tip from Hardwood Products Company or from Hain Diagnostika).
- All centrifugation steps are carried out at room temperature (15–25°C).
- To collect a sample, scrape the swab firmly against the inside of each cheek 6 times. Air-dry the swab for at least 2 h after collection. Ensure that the person providing the sample has not consumed any food or drink in the 30 min prior to sample collection.

1. **Place buccal swab in a 2 ml microcentrifuge tube. Add 400 ul (cotton and DACRON swab) or 600 ul (Omni Swab) PBS to the sample.**
   The Omni Swab is ejected into the microcentrifuge tube by pressing the stem end towards the swab. Cotton or DACRON swabs are separated from the stick by hand or with scissors.
   If RNA-free genomic DNA is required, 4 ul of an RNase A stock solution (100 mg/ml) should be added to the sample prior to the addition of Buffer NL.

2. **Add 20 ul Proteinase K and 400 ul (cotton or DACRON swab) or 600 ul (Omni Swab) Buffer NL to the sample. Mix immediately by vortexing for 15 s.**
   In order to ensure efficient lysis, it is essential that the sample and Buffer NL are mixed immediately and thoroughly.
   **Note:** Do not add Proteinase K directly to Buffer NL.

3. **Incubate at 56°C for 10 min. Briefly centrifuge to remove drops from inside the lid.**

4. **Add 400 ul (cotton or DACRON swab) or 600 ul (Omni Swab) ethanol (96–100%) to the sample, and mix again by vortexing. Briefly centrifuge to remove drops from inside the lid.**

5. **Carefully apply 700 ul of the mixture from step 4 to the Mini-column (in a 2 ml collection tube) without wetting the rim, close the cap, and centrifuge at 6,000 x g (8,000 rpm) for 1 min. Place the Mini-column in a clean 2 ml collection tube, and discard the tube containing the filtrate.**
   Close each Mini-column in order to avoid aerosol formation during centrifugation.

6. **Repeat step 5 by applying up to 700 ul of the remaining mixture from step 4 to the Mini-column.**

7. **Carefully open the Mini-cColumn and add 500 ul Wash C Solution without wetting the rim. Close the cap and centrifuge at 6,000 x g (8,000 rpm) for 1 min. Place the Mini-column in a clean 2 ml collection tube, and discard the collection tube containing the filtrate.**

8. **Carefully open the Mini-column and add 500 ul Wash D Solution without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min. Continue directly with step 9, or to eliminate any chance of possible Wash D Solution carryover, perform step 8a, and then continue with step 9.**
   **Note:** Residual Wash D Solution in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains Wash D Solution, coming into contact with the Mini-column. Removing the Mini-column and collection tube from the rotor may also cause flow-through to come into contact with the Mini-column. In these cases, the optional step 8a should be performed.

8a. **(Optional): Place the Mini-column in a new 2 ml collection tube and discard the collection tube with the filtrate. Centrifuge at 20,000 x g (14,000 rpm) for 1 min.**

9. **Place the Mini-column in a clean 1.5 ml microcentrifuge tube. Elution the DNA with 150 ul Elution Buffer or distilled water. Incubate at room temperature for 1 min, and then centrifuge at 6,000 x g (8,000 rpm) for 1 min.**
   Elution with 100 ul Elution Buffer increases the final DNA concentration in the eluate significantly, but may slightly reduce the overall DNA yield. Elution with volumes of less than 100 ul is not recommended as the overall DNA yield decreases dramatically.
   A second elution step with the same 150 ul eluate containing the DNA will increase yield significantly. However this is not recommended when using the eluate for PCR.
   One buccal swab typically yields 0.5–3.5 ug of DNA in 150 ul of buffer (3–23 ng/ul), with *A*260/*A*280 ratios of 1.7–1.9 (measured in water).

## Protocol for Buccal Swab
### Using a Vacuum Manifold

**Please read "Important Notes" on pages 6 before starting.**

1. **Place buccal swab in a 2 ml microcentrifuge tube. Add 400 ul (cotton and DACRON swab) or 600 ul (Omni Swab) PBS to the sample.**
   The Omni Swab is ejected into the microcentrifuge tube by pressing the stem end towards the swab. Cotton or DACRON swabs are cut from the stick by hand or with scissors.
   If RNA-free genomic DNA is required, 4 ul RNase A stock solution (100 mg/ml) should be added to the sample prior to the addition of Buffer NL.
2. **Add 20 ul Proteinase K and 400 ul (cotton or DACRON swab) or 600 ul (Omni Swab) of Buffer NL to the sample. Mix immediately by vortexing for 15 s.**
   In order to ensure efficient lysis, it is essential that the sample and Buffer NL are mixed immediately.
   **Note:** Do not add Proteinase K directly to Buffer NL.
3. **Incubate at 56°C for 10 min. Briefly centrifuge to remove drops from inside the lid.**
4. **Add 400 ul (cotton or DACRON swab) or 600 ul (Omni Swab) ethanol (96–100%) to the sample, and mix again by vortexing.**
5. **Insert the Mini-column on the vacuum manifold.**
6. **Apply the mixture from step 4 to the Mini-column. Switch on the vacuum pump to draw the lysate through the Mini-column. After the lysate has passed through the Mini-column, switch off the vacuum pump.**
7. **Add 750 ul Wash C Solution into the Mini-column. Switch on the vacuum pump to draw Wash C Solution through the Mini-column. Switch off the vacuum pump.**
8. **Add 750 ul Wash D Solution into the Mini-column. Switch on the vacuum pump to draw Wash D Solution through the Mini-column. Switch off the vacuum pump.**
9. **Close the lid of the Mini-column, remove it from the vacuum manifold. Place the Mini-column into a clean 2 ml collection tube and centrifuge at 20,000 x g (14,000 rpm) for 1 min to dry the membrane completely.**
10. **Place the Mini-column in a clean 1.5 ml microcentrifuge tube. Elute the DNA with 150 ul Elution Buffer or distilled water. Incubate at room temperature for 1 min, and then centrifuge at 6,000 x g (8,000 rpm) for 1 min.**
    Elution with 100 ul Elution Buffer increases the final DNA concentration in the eluate significantly, but may slightly reduce the overall DNA yield. Elution with volumes of less than 100 ul is not recommended as overall DNA yield decreases dramatically.
    A second elution step with the same 150 ul eluate containing the DNA will increase yield significantly. However this is not recommended when using the eluate for PCR.
    One buccal swab typically yields 0.5–3.5 ug DNA in 150 ul buffer (3–23 ng/ul), with *A*260/*A*280 ratios of 1.7–1.9 (measured in water).

## Ordering Information

| Products | | Contents | Cat. No. |
|---|---|---|---|
| RNA Stabilization Reagent | | Tube (50 x 1.5 ml) | 3502 |
| | | Tube (20 x 5 ml) | 3205 |
| | | 100 ml | 3100 |
| | | 250 ml | 3250 |
| Plasmid Purification Mini Kit | | | |
| | (200) for negative strain | 200 preps | 5112 |
| | (200) for positive strain | 200 preps | 7112 |
| Plasmid Purification Midi Kit | (10) | 10 preps | 6101 |
| | (50) | 50 preps | 6105 |
| | (100) | 100 preps | 6110 |
| Plasmid Purification Maxi Kit | (6) | 6 preps | 7106 |
| | (24) | 24 preps | 7124 |
| | (50) | 50 preps | 7150 |
| Gel Extraction Kit | (50) | 50 preps | 5215 |
| | (200) | 200 preps | 5212 |
| Highcon Gel Extraction Kit | (50) | 50 preps | 2215 |
| | (200) | 200 preps | 2212 |
| Bead Type (NaI) Gel Extraction Kit | (200) | 200 preps | 1232 |
| | (400) | 400 preps | 1234 |
| | (600) | 600 preps | 1236 |
| PCR Purification Kits | (50) | 50 preps | 5315 |
| | (200) | 200 preps | 5312 |
| Highcon PCR Purification Kit | (50) | 50 preps | 2315 |
| | (200) | 200 preps | 2312 |
| DNA Clean-up Kits | (50) | 50 preps | 1415 |
| | (200) | 200 preps | 1412 |
| Genomic Blood Spin Mini Kit | (50) | 50 preps | 1515 |
| | (200) | 200 preps | 1512 |
| Genomic Blood Spin Midi Kit | (20) | 20 preps | 6520 |
| | (50) | 50 preps | 6550 |
| | (100) | 100 preps | 6500 |
| Genomic Blood Spin Maxi Kit | (6) | 6 preps | 7506 |
| | (24) | 24 preps | 7524 |
| | (50) | 50 preps | 7550 |

## Ordering Information

| Products | | Contents | Cat. No. |
|---|---|---|---|
| Genomic Cell / Tissue Spin Mini Kit | (50) | 50 preps | 1545 |
| | (200) | 200 preps | 1542 |
| Genomic Cell / Tissue Spin Midi Kit | (20) | 20 preps | |
| | (50) | 50 preps | |
| | (100) | 100 preps | |
| Genomic DNA Isolation, Flexible | | 100 Isolation | 1521 |
| | | 500 Isolation | 1525 |
| | | 10 ml x 100 Isolation | |
| Apoptotic DNA Ladder Kit | | 50 preps | 2505 |
| 96 PCR Purification Kit | | | |
| 4 x 96 plates(binding, elution), buffer, tape | | | 4304 |
| 25 x 96 plates(binding, elution), buffer, tape | | | 4325 |
| 50 x 96 plates(binding, elution), buffer, tape | | | 2 x 4325 |
| 96 Plasmid Purification Kit | | | |
| 4 x 96 plates(clarification, binding, elution), buffer, tape | | | 4104 |
| 25 x 96 plates(clarification, binding, elution), buffer, tape | | | 4125 |
| 96 Genomic Blood Spin Kit | | | |
| 4 x 96 plates(binding, elution), buffer, tape | | | |
| 25 x 96 plates(binding, elution), buffer, tape | | | |
| 96 Genomic Cell / Tissue Spin Kit | | | |
| 4 x 96 plates(binding, elution), buffer, tape | | | |
| 25 x 96 plates(binding, elution), buffer, tape | | | |
| 50 x 96 plates(binding, elution), buffer, tape | | | 2 x |


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