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Product Information

GenElute™ 96 Well Blood Genomic DNA Purification Kit

Catalog Numbers **BLD9601, BLD9604**

TECHNICAL BULLETIN

Product Description

Sigma's GenElute 96 Well Blood Genomic DNA Purification Kit allows for high throughput purification of pure genomic DNA from whole blood, buffy coat, or cultured cells. The kit contains the advantages of silica membrane technology and eliminates the need for expensive resins, alcohol precipitations and hazardous compounds such as phenol and chloroform.

The starting materials are lysed at room temperature in a lysis buffer which contains chaotropic salts in the presence of Proteinase K. The addition of ethanol ensures the appropriate conditions to bind the DNA to the silica membrane of the Blood Binding Plate. After washing the bound DNA to remove contaminants, the pure genomic DNA is eluted in the Blood Elution Solution included in the kit.

Storage

Note: Store Blood Binding Plates, Catalog Number B9188, at 2-8 °C

Store other components of the GenElute 96 Well Blood Genomic DNA Purification Kit at room temperature. If any regent forms a precipitate, warm at 30-40 °C until the precipitate dissolves and allow to cool to room temperature before use. All reagents are stable for at least one year when stored properly.

Reagents and Equipment Required but Not Provided

- GenElute Vacuum Manifold, Cat No. VM01
- Vacuum Source (vacuum of 200-400mbar)
- GenElute Vacuum Regulator, Cat No. VM02 (recommended)
- 95-100% Ethanol, Cat No. E7148, E7023 or 459836

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Blood Lysis Solution and Blood Wash Buffer 1 contain guanidinium hydrochloride which is an irritant so gloves and safety glasses should be worn.

Proteinase K is an irritant to eyes, respiratory system, and skin. It may also cause sensitization by inhalation. Gloves and safety glasses should be worn.

Reagents Provided	Catalog Number	BLD9601 1x 96 Preps	BLD9604 4x96 Preps
Blood Lysis Solution	L7295	40 mL	125 mL
Blood Wash Buffer 1	W3895	125 mL	2 x 300 mL
Blood Wash Buffer 2, Concentrate	W3770	100 mL	4 x 100 mL
Blood Elution Solution	E5661	50 mL	125 mL
Blood Proteinase K	B9563	75 mg	4 x 75mg
Blood Proteinase Buffer	B9063	3.6 mL	15 mL
Blood Binding Plates	B9188	1 Each	4 Each
Wash Plate	W3270	1 Each	4 Each
Lysis Blocks	L7420	1 Each	4 Each
Rack of Strip Tubes	R3283	1 Each	4 Each
Tubes, 2 mL	T7955	4 Each	16 Each
Tubes, 15 mL	T8080	8 Each	32 Each

Preparation Instructions
Before beginning the procedure, complete the following:

- Reconstitute Blood Proteinase K by adding 3.35 mL of Blood Proteinase Buffer to each 75 mg vial of Proteinase K. The resulting solution should be stored at -20 °C for up to 6 months.

- Blood Wash Buffer 2, Concentrate: Add 400 mL of 96-100 % ethanol to each 100 mL bottle of Blood Wash Buffer 2, Concentrate. Mark the label of the bottle to indicate that ethanol is added. After each use, tightly cap the diluted Blood Wash Buffer 2 to prevent ethanol evaporation. This buffer is stable for up to one year at room temperature.

Vacuum Manifold Use

Establish a reliable vacuum source for the GenElute Vacuum Manifold. The manifold may be used with a vacuum pump, house vacuum, or water aspirator. A vacuum of 200-400 mbar (pressure difference) is recommended. The use of a GenElute Vacuum Regulator (VM02) is also recommended. Alternatively, adjust vacuum during the purification such that the sample flows through the column with a rate of 1-2 drops per second. To check the vacuum in advance, it is recommended to place a Blood Binding Plate on top of the vacuum lid. Seal the Binding Plate with common self-adhering foil. Close the manifold's valve and check and adjust the vacuum pressure.

Procedures

DNA Isolation using a vacuum manifold

1. **Lyse Sample**

   Add 200 µL blood, equilibrated to room temperature, and 25 µL Blood Proteinase K to each well of the Lysis Block.

   Add 200 µL Blood Lysis Buffer to each well and, mix 3 times by pipetting up and down. Incubate the samples at least 10 min at room temperature or incubate with shaking for 10 min at 1250 rpm at room temperature.

2. **Prepare the GenElute Vacuum Manifold**
   a. The spacers are inserted notch side up into the grooves located on the short sides of the manifold.
   b. The waste container is placed into the manifold base.
   c. The Wash Plate is placed onto the spacers.
   d. Close the manifold with the manifold lid.
   e. Insert Blood Binding Plate into the manifold lid.

3. **Adjust DNA binding conditions.** Add 200 µL 96-100% ethanol to each well of the Lysis Block and mix at least 2 times.

4. **Load samples.** Transfer the samples from the Lysis Block to the Blood Binding Plate.

   Note: To minimize cross contamination, do not wet the rims of the individual wells while dispensing the samples.

5. **Overlay with Blood Wash Buffer 2.** Overlay crude lysate on the Blood Binding Plate **slowly** (50 µL/s) with 150 µL Blood Wash Buffer 2.

6. **Bind DNA to silica membrane.** Apply vacuum until all lysates have passed through the wells of the Blood Binding Plate (-0.2 bar; 5min). Release the vacuum.

7. **Wash silica membrane**. Add 600 µL Blood Wash Buffer 1 to each well of the Blood Binding Plate. Apply vacuum (-0.2 bar; 3 min) until all Blood Wash Buffer 1 has passed through the wells of the Blood Binding Plate. Release the vacuum.

   Add 900 µL Blood Wash Buffer 2 to each well of the Blood Binding Plate. Apply vacuum (-0.2 bar; 1 min) until all Blood Wash Buffer 2 has passed through the wells of the Blood Binding Plate. Release the vacuum.

   Add 900 µL Blood Wash Buffer 2 to each well of the Blood Binding Plate. Apply vacuum (-0.2 bar; 1 min) until all Blood Wash Buffer 2 has passed through the wells of the Blood Binding Plate. Release the vacuum.

8. **Remove Wash Plate.** After the final wash, close the valve, release the vacuum and remove the Blood Binding Plate. Place the Blood Binding Plate on a clean paper towel to remove residual ethanol containing wash buffer. Remove the manifold lid, Wash Plate, and waste container from the GenElute Vacuum Manifold.

9. **Dry silica membrane.** Remove any residual Wash Solution from the Blood Binding Plate. If necessary, tap the outlets of the Blood Binding Plate on clean paper sheets (supplied with the Wash Plate) or soft tissue until no drops come out. Insert the Blood Binding Plate into the lid and close the manifold. Apply vacuum of (-0.6 bar) for at least 10 minutes to dry the membrane completely to eliminate any traces of ethanol.

   Note: The ethanol in Blood Wash Buffer 2 may inhibit enzymatic reactions if not removed completely before DNA elution.

10. **Elute DNA.** Insert spacers "Microtube rack" into the GenElute Vacuum Manifold's short sides. Place a Rack of Tube Strips onto the spacer. Close the vacuum manifold and place the Blood Binding Plate on top. Dispense 50-200 µL Blood Elution Solution onto the membrane. Incubate for 5 minutes at room temperature. Apply vacuum for elution (-0.6 bar; 1 min).

    Finally, close the Tube Strips with Cap Strips for storage.

    Note: Preheat Blood Elution Solution to 70 °C to increase yield.

DNA Isolation using a centrifuge.
Centrifuges that are compatible with the GenElute 96 Well Blood Genomic DNA Purification Kit should be able to pick up a swing out rotor which is capable of accommodating the Binding Plate/Square-well Block sandwich (bucket height is 85 mm) and reaches acceleration of 5,600 – 6,000 x *g*.

1. **Lyse Sample.**
   Add 200 µL blood, equilibrated to room temperature, and 25 µL Blood Proteinase K to each well of the Lysis Block. Add 200 µL Blood Lysis Buffer to each well and, mix 3 times by pipetting up and down. Incubate the samples at least 10 min at room temperature or incubate with shaking for 10 min at 1250 rpm at room temperature.

2. **Adjust DNA binding conditions.**
   Add 200 µL 96-100% ethanol to each well of the Lysis Block and mix at least 5 times by pipetting up and down.

3. **Load samples.**
   Transfer the samples from the Lysis Block to the Blood Binding Plate.

Note: To minimize cross contamination, do not wet the rims of the individual wells while dispensing the samples.

4. **Bind DNA to silica membrane.**
   Place the Binding Plate on a Square-well Block and centrifuge at 5,600-6,000 x *g* for 3-5 min. Discard flow through.

5. **Wash silica membrane**.
   Add 600 µL Blood Wash Buffer 1 to each well of the Blood Binding Plate. Place the Binding Plate on a Square-well Block and centrifuge at 5,600-6,000 x *g* for 3 min. Discard flow through.

   Add 900 µL Blood Wash Buffer 2 to each well of the Blood Binding Plate. Place the Binding Plate on a Square-well Block and centrifuge at 5,600-6,000 x *g* for 3 min. Discard flow through.

   Add 900 µL Blood Wash Buffer 2 to each well of the Blood Binding Plate. Place the Binding Plate on a Square-well Block and centrifuge at 5,600-6,000 x *g* for 10 min to completely dry the silica membrane. Discard flow through.

Note: A separate drying step is not required.

6. **Elute DNA.**
   Place the Binding Plate on top of a rack of tube strips, add 50 to 200 µL Blood Elution Solution, let it sit for 5 min and centrifuge at 5,600-6,000 x *g* for 3 min.

Note: Preheat Blood Elution Solution to 70 °C to increase yield.

Troubleshooting Guide

Problem	Cause	Solution
Poor DNA quality or yield	Low Concentration of leukocytes in the whole blood sample	Prepare buffy coat from the blood sample
	Incomplete cell lysis	Sample not thoroughly mixed with Blood Lysis Buffer/Blood Proteinase K. Use of a shaker is recommended for optimal results.
		Blood Proteinase K digestion not optimal. Do not add Blood Proteinase K directly to Blood Lysis Buffer.
		Increase incubation time. Incubate for at least 10 min at RT.
	Reagents not reconstituted properly	Add the indicated volume of Blood Proteinase Buffer to the Blood Proteinase K vial and 96-100% ethanol to Blood Wash Buffer 2, Concentrate and mix.
	Suboptimal elution	Elution efficiencies decrease dramatically if elution is done with buffers with pH < 7.0. Use slightly alkaline elution buffer like Blood Elution Solution (pH 8.5)
		Be sure that all of the Blood Elution Solution gets into contact with the silica membrane. No drops should stick to the walls of the columns.
	Kit Storage	Store aliquots of the reconstituted Blood Proteinase K at -20 °C
		Store other kit components at room temperature. Storage at low temperatures may cause salt precipitation.
		Keep bottles tightly closed in order to prevent evaporation or contamination.
Clogging of Blood Binding Plate	Clotting of blood samples	If blood samples are too old and clotting occurs, the Blood Binding Plate may become clogged. Check for blockage of Blood Binding Plate visually or automatically, and remove supernatant. Increase time and strength for vacuum processing. Whole blood can be stored for several weeks at 4 °C. Freeze samples at -20 °C if blood should be stored for a longer period.
Suboptimal performance of DNA in downstream experiments	Carryover of ethanol	Be sure to remove all of the ethanolic Blood Wash Buffer 2 after the final washing step. Dry the Blood Binding Plate for at least 10 min with maximum vacuum or by 10 min centrifugation
		Following the final wash step place Blood Binding Plate in an incubator for 10 min at 70 °C to evaporate ethanol
Vacuum manifold	Vacuum pressure is not sufficient	Check if the vacuum manifold lid fits tightly on the manifold base if vacuum is turned on.
		Make sure that pump works properly and that any in-line filters are not blocked.

Buffers	Buffer volumes are not enough	Buffers are delivered in sufficient, but limited amounts. Calculate the required buffer volumes and pour an additional amount of 10% into the reservoirs.
		Do not return unused buffer from reservoir to the bottle to avoid contamination.
Contamination of genomic DNA and RNA	RNA carryover	Add 10 µL (25 mg/ml) RNase A (Cat. No. R6513) to the sample after the incubation of the Lysis step, as recommended for working with fresh, unfrozen cells.

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