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Product Information

GenElute™ 96 Well Tissue Genomic DNA Purification Kit

Catalog Number **G1N9604**

TECHNICAL BULLETIN

Product Description

Sigma's GenElute 96 Well Tissue Genomic DNA Purification Kit provides a simple and convenient method to isolate pure genomic DNA from a variety of cultured cells and tissues. The kit contains the advantages of silica binding and eliminates the need for expensive resins, alcohol precipitation and hazardous organic compounds such as phenol and chloroform.

The starting material is lysed by incubation in a solution containing SDS and Proteinase K. Once lysis is complete, chaotropic salts are then added to further promote solubilization of macromolecules. The next step is to add ethanol which ensures that the appropriate binding conditions are created to bind the DNA to the silica membrane. Once the DNA is bound to the silica membrane several wash buffers are used to remove contaminants. The final step is to elute pure genomic DNA in the Tissue Elution Buffer included in the kit.

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.

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.

Tissue Binding Buffer and Tissue Wash Buffer 1 contain guanidinium hydrochloride which is an irritant so gloves and safety glasses should be worn.

Storage

Store the components of the GenElute 96 Well Total 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 Provided	Catalog Number	G1N9604 4x96 Preps
Tissue Lysis Buffer	L7545	100 mL
Tissue Binding Buffer	B9313	100 mL
Tissue Wash Buffer 1	W4145	2 x 125 mL
Tissue Wash Buffer 2, Concentrate	W4020	2 x 100 mL
Tissue Elution Buffer	E5786	100 mL
Tissue Proteinase K	K4644	4 x 75 mg
Tissue Proteinase Buffer	B9438	15 mL
Tissue Binding Plates	T8205	4 Each
Round-well Blocks	R3158	4 Each
Square-well Blocks	S9326	4 Each
Wash Plates	W3270	4 Each
Rack of Tube Strips	R3283	4 Each
Cap Strips	S9826	48 Each
Self-adhering PE Foil	S9201	10 Each

Reagents and Equipment Required but Not Provided

- GenElute 96 Vacuum Manifold, Cat No. VM01
- Vacuum Source (vacuum of 200-400 mbar)
- GenElute Vacuum Regulator, Cat No. VM02 (recommended)
- 95-100% Ethanol, Cat No. E7148, E7023 or 459836
- Centrifuge capable of 5,600-6,000 *x* g with bucket height 85nm
- Incubator or oven

Preparation Instructions

Before beginning the procedure, complete the following:

1. Reconstitute Tissue Proteinase K by adding 2.6 mL of Tissue Proteinase Buffer to each 75 mg vial of Proteinase K. The resulting solution should be stored at -20 °C for up to 6 months.
2. Tissue Wash Buffer 2, Concentrate
   Add 400 mL of 96-100 % ethanol to each 100 mL bottle of Tissue Wash Buffer 2, Concentrate. Mark the label of the bottle to indicate that ethanol has been added. After each use, tightly cap the diluted Tissue Wash Buffer 2 to prevent ethanol evaporation.
3. Set incubator or oven to 56 °C
4. Preheat Tissue Elution Buffer to 70 °C

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 Tissue 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.

Procedure

DNA Isolation using vacuum manifold

Prepare the 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. Place the Wash Plate on the spacers.
d. Close the manifold with the manifold lid
e. Place a Tissue Binding Plate on top of the manifold.

1. **Lyse Sample**

   For each sample, cut up to two 0.5 cm pieces (20 mg) of mouse tail into appropriate lysis tubes or plates. If preparing DNA from rat tails, one 0.5 cm piece is sufficient. Tissue samples should not exceed 20 mg, cultured cells and bacterial should not exceed 10^6 cells.

   Prepare a Proteinase K working solution: For each sample, mix 25 µL Proteinase K with 180 µL Tissue Lysis Buffer and vortex. Transfer 200µL of the Proteinase K/Tissue Lysis Buffer solution to each well of the Round-well Block containing the samples. Close with Cap Strips and mix by shaking vigorously for 10-15 seconds. Spin briefly for 15 seconds at 1,500 x *g* to collect all samples at the bottom of the wells.

   Note: The samples must be completely covered with solution. Never prepare the Proteinase K working solution more than 15 min before addition to the samples because the Proteinase K will self digest without substrate.

   Incubate the Round-well Block containing the samples at 56 °C for at least 6 hours; bacterial cells might require a pre-lysis with, e.g., lysozyme. If working with mammalian cells the incubation step can be reduced to 10 minutes. For optimal lysis, mix occasionally during incubation. Place a weight on top of the Round-well Block in order to prevent the Cap Strips from popping off.

   Note: After lysis, set the incubator to 70 °C for the membrane drying step.

Centrifuge the Round-well Block for 15 seconds at 1,500 x *g* to collect any condensate from the Cap Strips. Remove Cap Strips.

Note: Residual hair and/or bones in the lysate can be removed by centrifugation for 2 minutes at 5,600-6,000 x *g* and transfer the supernatant to a new Round-well Block (not supplied with the kit).

2. **Adjust DNA binding conditions.** Add 200 µL Tissue Binding Buffer and 200 µL 96-100% ethanol to each sample. Close the individual wells with new Cap Strips. Shake vigorously for 10-15 seconds to mix. Spin for 10 seconds at 1,500 x *g* to collect any sample from the Cap Strips.

   Note: Ethanol and Tissue Binding Buffer can be premixed before addition to the samples, if the mixture is to be used during the next 3 months. Never centrifuge at faster speed or longer periods of time as this will precipitate the DNA.

3. **Load samples.** Remove the Cap Strips and transfer the lysates resulting from the previous step carefully from the Round-well Block into the wells of the Tissue Binding Plate.

   Note: Use an eight-channel pipetting device with extra long tips capable of holding more than 650 µL to transfer the lysate from the Round-well Block to the Tissue Binding Plate.

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

5. **Wash silica membrane**. Add 600 µL Wash Buffer 1 to each well of the Tissue Binding Plate. Apply vacuum (-0.2 bar; 5 min) until all Wash Buffer has passed through the wells of the Tissue Binding Plate. Release vacuum.

   Add 900 µL Tissue Wash Buffer 2 to each well of the Tissue Binding Plate. Apply vacuum (-0.2 bar; 5 min) until all Tissue Wash Buffer 2 has passed through the wells of the Tissue Binding Plate. Release vacuum.

   Add 900 µL Tissue Wash Buffer 2 to each well of the Tissue Binding Plate. Apply vacuum (-0.2 bar; 5 min) until all Tissue Wash Buffer 2 has passed through the wells of the Tissue Binding Plate. Release vacuum.

6. **Remove Wash Plate.** After the final washing step, close the valve, release the vacuum and remove the Tissue Binding Plate. Place the Tissue Binding Plate on a clean paper towel to remove residual ethanol containing wash buffer. Remove manifold lid, Wash Plate, and waste container from the vacuum manifold.

   Dry silica membrane. Insert the Tissue Binding Plate into the lid and close the manifold. Apply maximum vacuum (at least -0.6 bar) for 10 minutes to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

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

   Finally, release the vacuum.

7. **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 Tissue Binding Plate on top. Dispense 100 µL preheated Tissue Elution Buffer onto the membrane. Incubate for 3 min at room temperature. Apply vacuum for elution (-0.4 bar; 2 min). Release the vacuum and repeat the elution step

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

   Centrifuge Rack of Tube Strips shortly to collect all samples at the bottom of the Tube Strips.

DNA Isolation using a centrifuge

1. **Lyse Sample**

   For each sample, cut up to two 0.5 cm pieces (20 mg) of mouse tail into appropriate lysis tubes or plates. If preparing DNA from rat tails, one 0.5 cm piece is sufficient. Tissue samples should not exceed 20 mg, cultured cells and bacterial should not exceed 10^6 cells.

   Prepare a Proteinase K working solution: For each sample, mix 25 µL Proteinase K with 180 µL Tissue Lysis Buffer and vortex. Transfer 200µL of the Proteinase K/Tissue Lysis Buffer solution to each well of the Round-well Block containing the samples. Close with Cap Strips and mix by vigorously shaking for 10-15 seconds. Spin briefly for 15 seconds at 1,500 x *g* to collect all samples at the bottom of the wells.

   Note: The samples must be completely covered with solution. Never prepare the Proteinase K working solution more than 15 min before addition to the samples because the Proteinase K will self digest without substrate.

   Incubate the Round-well Block containing the samples at 56 °C for at least 6 hours; bacterial cells might require a pre-lysis with, e.g., lysozyme. If working with mammalian cells the incubation step can be reduced to 10 minuets. For optimal lysis, mix occasionally during incubation. Place a weight on top of the Round-well Block in order to prevent the Cap Strips from popping off.

   Note: After lysis, set the incubator to 70 °C for the membrane drying step.

   Centrifuge the Round-well Block for 15 seconds at 1,500 x *g* to collect any condensate from the Cap Strips. Remove Cap Strips.

   Note: Residual hair and/or bones in the lysate can be removed by centrifugation for 2 minutes at 5,600-6,000 x g and transfer of the supernatant to a new Round-well Block (not supplied with the kit)

2. **Adjust DNA binding conditions.** Add 200 µL Tissue Binding Buffer and 200 µL 96-100% ethanol to each sample. Close the individual wells with new Cap Strips. Shake vigorously for 10-15 seconds to mix. Spin for 10 seconds at 1,500 x *g* to collect any sample from the Cap Strips.

   Note: Ethanol and Tissue Binding Buffer can be premixed before addition to the samples, if the mixture is to be used during the next 3 months. Never centrifuge at higher g-forces or for longer periods as DNA will precipitate.

3. **Load samples.** Place the Tissue Binding Plate on a Square-well Block**.** Remove the Cap Strips and transfer the lysates resulting from the previous step carefully from the Round-well Block into the wells of the Tissue Binding Plate. After transfer, seal the openings of the plate with Self-adhering PE Foil.

   Note: Use an eight-channel pipetting device with extra long tips capable of holding more than 650 µL to transfer the lysate from the Round-well Block to the Tissue Binding Plate.

4. **Bind DNA to silica membrane.** Place the Square-well Blocks with Tissue Binding Plates onto the centrifuge carriers and insert into the rotor buckets. Centrifuge at 5,600-6,000 x *g* for 10 minutes.

Note: The use of a second binding plate placed on a Square-well Block avoids the need to balance the centrifuge

Note: The lysates will typically pass through the silica membrane within a few minutes. The centrifugation process can be extended to 20 min, if the lysates have not passed completely.

5. **Wash silica membrane**. Remove the Self-adhering PE Foil and add 500 µL Wash Buffer 1 to each of the Tissue Binding Plate. Seal the plate with a new Self-adhering PE Foil and centrifuge again at 5,600-6,000 x *g* for 2 minutes.

   Remove the Self-adhering PE Foil and add 700 µL Tissue Wash Buffer 2 to each well of the Tissue Binding Plate. Seal the plate with new Self-adhering PE Foil and centrifuge again at 5,600-6,000 x *g* for 4 minutes.

   Note: This step removes as much of the buffers containing ethanol as possible by centrifugation

6. **Dry silica membrane.** Remove the Self-adhering PE Foil and place the Tissue Binding Plate on an opened Rack of Tube Strips. Place it in an incubator for 10 min at 70 °C to evaporate residual ethanol. Removal of ethanol by evaporation at 70 °C is more efficient than prolonged centrifugation.

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

7. **Elute DNA.** Dispense 100 µL preheated Tissue Elution Buffer to each well of the Tissue Binding Plate. Dispense the buffer directly onto the membrane. Incubate at room temperature for 1 minute. Centrifuge at 5,600-6,000 x *g* for 2 minutes. Repeat elution step once.

   Remove Tissue Binding Plate from the Rack of Tube Strips by lifting the plate at one side carefully as the Tubes Strips may stick to the outlets of the Tissue Binding Plate. If eluting in small volume tubes is desired, place a 96 PCR plate (not supplied) on top of a Round-well Block or a Rack of Tube Strips and elute into the PCR plate.

Troubleshooting Guide

Problem	Cause	Solution
No or poor DNA yield	Incomplete cell lysis	Sample not completely submerged during heat incubation. Cut samples into small pieces. Mix well. Be sure that the samples are fully submerged in Tissue Lysis Buffer/ Tissue Proteinase K mixture. Incubate until the samples are completely lysed.
		Tissue Lysis Buffer and Tissue Proteinase K premixed more than 15 min before addition to the substrate. Proteinase K tends to self digest under optimal reaction conditions in Buffer T1 without substrate.
	Reagents not reconstituted properly	Prepare Tissue Wash Buffer 2 and Tissue Proteinase K solution according to instructions. Add Tissue Binding Buffer and ethanol to the lysates before loading them to the wells of the Tissue Binding Plate.
	Suboptimal elution	Elution efficiencies decrease dramatically if elution is done with buffers with pH < 7.0. Use slightly alkaline elution buffer like Tissue Elution Solution (pH 8.5)
		Preheat Tissue Elution Buffer to 70 °C before elution. Apply Tissue Elution Buffer directly onto the center of the silica membrane.

Clogged wells	Too much starting material	Repeat the procedure, using two mouse tail sections of maximally 4 - 6 mm length. If processing rat tails, one 0.5 cm-long tail tip section is sufficient.
	Hair or bones left in the lysate after step 2	Centrifuge the Round-well Block for 3 min at 5,600 - 6,000 x *g*. Transfer lysates to a new Round-well Block without disturbing the debris pellet.
	Incomplete passage of lysate in step 4	If no more than 300-500 µL of lysate is remaining in the columns, continue with step 5. Through the addition of Tissue Wash Buffer 1 the sample is diluted and thus the sample will pass the column more easily.
Poor performance of genomic DNA in enzymatic reactions	Carryover of ethanol	After washing with Tissue Wash Buffer 2 centrifuge ≥ 4 min at 5,600-6,000 x *g* in order to remove ethanolic Tissue Wash Buffer 2 completely and evaporate residual ethanol by incubating the Tissue Binding Strips / Plate at 70 °C for 10 min.
		Increase vacuum drying time to 15 min.
RNA contamination	RNA in sample	If DNA free of RNA is desired, cool down to room temperature after lysis incubation and add 20 µL of an RNase A solution, 20 mg/ml; Catalog Number R6513. Incubate for 15 min with moderate shaking.

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GS,SW,PHC 07/17-1