GenElute™ MAMMALIAN GENOMIC DNA KIT
Product Number **GE-G1-N**

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ProductInformation

TECHNICAL BULLETIN

Product Description

Sigma's GenElute™ Mammalian Genomic DNA Kit provides a simple and convenient way to isolate pure genomic DNA from a variety of cultured cells and tissues. The GenElute kit combines the advantages of silica binding with a microspin format, and eliminates the need for expensive resins, alcohol precipitation, and hazardous organic compounds such as phenol and chloroform. Cells or tissues are lysed in a chaotropic salt-containing buffer to insure thorough denaturing of macromolecules. Addition of ethanol causes DNA to bind when the lysate is spun through a silica membrane in a microcentrifuge tube. After washing to remove contaminants, DNA is eluted in 200 µl of Buffer SE (10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0).

Expected yields of genomic DNA will vary depending on the amount and type of starting material used (for example, 15-25 µg of RNase A-treated DNA can be isolated from 2 x 10^6 HeLa cells in less than one hour). DNA purified with the GenElute kit has an A_{260}/A_{280} ratio between 1.7 and 1.9 and can be up to 50 kb in length. This DNA is ready for downstream applications such as restriction endonuclease digests, PCR† amplification, Southern blots, and sequencing reactions.

Reagents Included

Sufficient for 50 isolations

- PBS buffer, Product No. P3980 — 15 ml
- Buffer STL, Product No. B6678 — 10 ml
- Buffer SL, Product No. B8803 — 12 ml
- Buffer SW, 5X Concentrate, Product No. B6553 — 12 ml
- Buffer SE, Product No. B6803 — 22 ml
- Proteinase K, Product No. P2308 — 25 mg
- RNase Solution, Product No. R6148 — 1.2 ml
- Nucleic Acid Binding Columns, Product No. C9471 — 50 each
- Collection Tubes, Product No. T7813 — 200 each

Reagents and Equipment Required But Not Provided
(Sigma Product numbers are given where appropriate)

- 55°C water bath or shaking water bath
- 70°C water bath or heating block
- Pipet tips with aerosol barrier (recommended)
- Wide bore pipet tips (1000 µl capacity)
- 1.5 ml microcentrifuge tube for lysis
- Microcentrifuge (2 ml tube rotor equipped)
- Ethanol, 95%. Product No. E7148 or 100%, Product No. E7023
- Molecular biology grade water, Product No. W4502

Precautions and Disclaimer

The GenElute™ Mammalian Genomic DNA Kit is for laboratory use only. Not for drug, household or other uses. Buffer SL contains a chaotropic salt, which is an irritant. Wear gloves, safety glasses, and suitable protective clothing when handling this solution or any reagent provided with the kit. See the Materials Safety Data Sheet (MSDS).

Storage

Store the kit at room temperature. If any kit reagent forms a precipitate, warm at 70°C until the precipitate dissolves and allow to cool to room temperature before use.

Preparation Instructions

Before beginning the procedure, do the following:

1. Preheat a water bath or shaking water bath to 55°C (for use with tissues).
2. Preheat a water bath or heating block to 70°C.
3. Buffer SW is supplied as a 5X concentrate. Dilute the concentrate with 48 ml of 95-100% ethanol. After each use, tightly cap the diluted Buffer SW to prevent the evaporation of ethanol.

4. Resuspend Proteinase K (25 mg) in 1.25 ml of water (Product No. W4502) to obtain a 20 mg/ml stock solution. This solution can be stored for several days at 2-8°C; for longer-term storage, the unused portion of the solution may be stored in aliquots (e.g., 5 x 200 µl) at –20°C until needed.

Procedure
All steps are carried out at room temperature unless otherwise noted.

I. Cultured Cell Preparation

1. Harvest cells

   a. Attached cell cultures. Release cells with trypsin. Pellet up to 5 x 10^6 cells for 5 minutes at 300 X g, then remove culture medium completely and discard. Continue to step 2.
   b. Suspension cell cultures. Pellet up to 5 x 10^6 cells for 5 minutes at 300 X g, then remove culture medium completely and discard. Continue with step 2.

      Note: Cells can be harvested, aliquoted into 1.5 ml microcentrifuge tubes and flash-frozen in liquid nitrogen, then stored at -70°C for several months before preparing DNA.

2. Resuspend pellet thoroughly in 200 µl PBS Buffer. Allow frozen cell pellet to thaw slightly before resuspending.

   Optional RNase treatment: If residual RNA is not a concern, continue with step 3.
   If RNA-free genomic DNA is needed, add 20 µl RNase Solution and incubate for 2 minutes at room temperature, then continue with step 3.

3. Add 20 µl proteinase K to the sample, then add 200 µl Buffer SL, vortex thoroughly (about 15 seconds), and incubate at 70°C for 10 minutes. Continue with section III, step 4.
   Note: A homogeneous mixture is essential for efficient lysis. Proteinase K must be added directly to each sample preparation every time. Do not combine Proteinase K and Buffer SL for storage.

   Note: If minimally sheared genomic DNA is desired in downstream applications, e.g. if using the end product for long amplification PCR, mix with gentle pipeting or inversion until homogeneous instead of vortexing.

II. Mammalian Tissue Preparation

1. Quickly mince and weigh a piece of fresh or frozen tissue. Allow frozen tissue to thaw slightly before slicing, but keep on ice to protect against degradation. Cutting tissue into small pieces enables more efficient lysis. Up to 25 mg tissue (or 10 mg spleen, due to the high number of cells per given mass) may be used per preparation. Transfer to a 1.5 ml micro-centrifuge tube and continue to step 2.

   Note: Tissue can be harvested, aliquoted into 1.5 ml microcentrifuge tubes and flash-frozen in liquid nitrogen, then stored at -70°C for several months before preparing DNA.

2. Add 180 µl Buffer STL, then add 20 µl Proteinase K to the tissue. Mix by vortexing then incubate the sample at 55°C until the tissue is completely digested and no particles remain. Vortex occasionally or use a shaking water bath. Digestion is usually complete in 2 to 4 hours. After digestion is complete vortex briefly.
   Note: If minimally sheared genomic DNA is desired in downstream applications, e.g. if using the end product for long amplification PCR, mix with gentle pipeting or inversion until homogeneous instead of vortexing.

   Optional RNase treatment: If residual RNA is not a concern, continue with step 3.
   If RNA-free genomic DNA is needed, add 20 µl RNase Solution and incubate for 2 minutes at room temperature, then continue with step 3.

3. Add 200 µl Buffer SL to the sample, vortex thoroughly (about 15 seconds), and incubate at 70°C for 10 minutes. Continue with section III, step 4.
   Note: A homogeneous mixture is essential for efficient lysis
   Note: If minimally sheared genomic DNA is desired in downstream applications, e.g. if using the end product for long amplification PCR, mix with gentle pipeting or inversion until homogeneous instead of vortexing.

III. DNA Isolation from Cultured Cells or Mammalian Tissues

4. Add 200 µl ethanol (95-100%) to the sample, and mix thoroughly by vortexing 5-10 seconds. A homogeneous solution is essential to this step.
   Note: If minimally sheared genomic DNA is desired in downstream applications, e.g. if using the end product for long amplification PCR, mix with gentle pipeting or inversion until homogeneous instead of vortexing.

5. Transfer entire contents of tube into a pre-assembled binding column/2 ml collection tube unit. Use a wide bore pipet tip to reduce shearing of DNA when transferring contents into the binding column. Centrifuge at ≥6500 X g for 1 minute. Discard the collection tube containing eluate and place the binding column in a new 2 ml collection tube.

6. Add 500 µl 1X Buffer SW‡ to the binding column and centrifuge for 1 minute at ≥6500 X g. Discard the collection tube containing eluate and place the binding column in a new 2 ml collection tube

   ‡Prior to first use, dilute Buffer SW 5X Concentrate with ethanol as described under Preparation Instructions.

7. Add another 500 µl 1X Buffer SW to the binding column, and centrifuge for 3 minutes at maximum speed (12,000-16,000 X g) to dry the binding column. Discard the collection tube containing eluate and place the binding column in a new 2 ml collection tube
   Note: The binding column must be free of ethanol before eluting DNA. Centrifuge the column an additional 1 minute at maximum speed if residual ethanol is noticed. Empty and re-use the collection tube for this additional spin.

8. Pipet 200 µl Buffer SE directly into the center of the binding column, and centrifuge for 1 minute at ≥6500 X g to elute the DNA.
   Note: To increase elution efficiency, incubate 5 minutes at room temperature after addition of Buffer SE, then centrifuge.

   Optional: A second elution can be collected by repeating step 8 with an additional 200 µl Buffer SE and eluting in a new 2 ml collection tube (provided) or in the same 2 ml collection tube as used for the first eluate.

For short-term storage of DNA, 2-8°C is recommended. For long-term storage of DNA, -20°C is recommended. Avoid freezing and thawing, which causes breaks in the DNA strand. Elution with Buffer SE will provide stability of the sample at these temperatures.

IV. Precipitation of DNA

The GenElute Mammalian Genomic DNA Kit is designed so that the DNA always remains in solution, thus avoiding resuspension problems. However, if you find it necessary to concentrate the DNA, ethanol precipitation in the presence of sodium acetate is recommended.[1]

Results

Analysis of Results

The concentration and quality of genomic DNA prepared with the GenElute kit can be determined by spectrophotometric analysis and agarose gel electrophoresis. Dilute the DNA in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0- 8.5 (TE buffer) and measure the absorbance at 260 and 280 nm using a quartz micro-cuvette. The absorbance should be between 0.1 and 1.0 (or within the linear range of your spectrophotometer). An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/ml of double-stranded DNA. The ratio of absorbance at 260 to 280 nm should be in the range of 1.7 to 1.9.

The size and quality of the DNA can be determined by agarose gel electrophoresis.[1] A gel containing 0.8% agarose (Product No. A9539) in 0.5X TBE Buffer (Product No. T6400) works well for the resolution of genomic DNA. The DNA can be visualized by staining with an intercalating dye such as ethidium bromide (Product No. E1510) and measured against a known DNA marker such as Lambda DNA EcoR I Hind III digest (Product No. D9281). The genomic DNA should migrate as a single, high molecular weight band with very little evidence of shearing. A more precise determination of the size of the DNA can be made by pulsed-field gel electrophoresis.[2]

Troubleshooting Guide

Problem	Cause	Solution
Binding column clogged	Sample size too large	Use fewer cells or smaller tissue samples. Increase g-force and/or spin longer until lysate passes through the binding column. Yield of genomic DNA may be reduced.
	Insufficient disruption	Extend proteinase K digestion at 55°C. To speed up lysis, cut tissue into smaller pieces and mix frequently during digestion to ensure more efficient lysis. Also, inverting the sample tube after proteinase K digestion will ensure a more homogeneous mixture.
Low Yield	State of sample	Yields will vary between different types of cells and tissues. Use cultures before they reach maximum density or as they become fully confluent, and harvest tissues as rapidly as possible. If samples are being stored for future use, flash-freezing in liquid nitrogen is recommended.
	Insufficient disruption	See above.
	Lysate with ethanol not homogeneous	To ensure a homogeneous solution, vortex 5-10 seconds before applying to the binding column. If minimally sheared genomic DNA is desired in downstream applications, e.g. if using the end product for long amplification PCR, mix with gentle pipeting or inversion until homogeneous instead of vortexing
	Incomplete elution	Check that DNA was eluted in 200 µl Buffer SE. A 5 minute incubation at room temperature after Buffer SE has been added to the binding column will improve yields with most types of material. Also, perform a second and third elution on the binding column using 200 µl Buffer SE for each elution.
	Ethanol addition omitted	Check that ethanol was added in step 4 before applying sample to the binding column in step 5.
	Residual ethanol	Ethanol from the final wash must be eliminated before eluting the DNA. A longer or additional spin, as recommended in step 7, is needed to dry the membrane. If eluate containing ethanol contacts the column, repeat the centrifugation step before eluting DNA.
	Buffer SW not diluted before use	Check that Buffer SW 5X concentrate was properly diluted with ethanol before use.
	Water used for elution instead of Buffer SE	Buffer SE is recommended for optimal yields and storage of end product. If water is used to elute DNA, confirm the pH is at least 7.0, to avoid acidic conditions, which would subject the DNA to acid hydrolysis when stored for long periods of time.

Troubleshooting Guide

Problem	Cause	Solution
Purity of DNA lower than expected: A_{260}/A_{280} ratio is too low	Sample diluted in water	Use either TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0-8.5) or 10 mM Tris-HCl, pH 8.0-8.5.
Purity of DNA lower than expected: A_{260}/A_{280} ratio is too high	RNA contamination	Include RNase A treatment step in future isolations.
Sheared DNA	Excessive manipulation	This kit was designed to eliminate precipitation and resuspension of the DNA, common steps found in other genomic DNA kits that can lead to shearing. All pipeting steps should be done as gently as possible. Wide-orifice pipet tips are recommended to help eliminate potential shearing. If minimally sheared genomic DNA is desired in downstream applications, e.g. if using the end product for long amplification PCR, mix with gentle pipeting or inversion until homogeneous instead of vortexing.
	Old sample or, sample has undergone repeated freeze/thaw cycles	Old starting material may yield degraded DNA in the eluate. Fresh cell and tissue preparations should be used immediately or frozen in liquid nitrogen and stored at -70°C until needed.
Inhibition of downstream applications	Ethanol carryover	After the final wash of the Binding Column (step 7) do not allow the eluate to contact the column. Re-spin the column, if necessary, by emptying the eluate from the collection tube and centrifuging the binding column for an additional 1 minute at maximum speed (12,000-16,000 X g).
	Salt carryover	Make sure that binding column is transferred to a new 2 ml collection tube before adding Buffer SW in step 6.

References

1. Sambrook, J., *et al.* Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989)

2. Birren, B. and Lai, E. Pulsed Field Gel Electrophoresis: A Practical Guide (Academic Press, San Diego, CA, 1993)

Related Products

- Agarose, Product No. A9539
- Ethanol, 100%, Product No. E7023
- Tris-EDTA Buffer (100X), Product No. T9285
- Ethidium Bromide, 10 mg/ml, Product No. E1510
- Microcentrifuge Tubes, 1.5 ml, Product No. T9661
- Taq DNA Polymerase, Product No. D1806
- AccuTaq™ LA DNA Polymerase, Product No. D8045
- KlenTaq™ LA Polymerase Mix, Product No. D6290
- Long PCR Core Kit, Product No. LCOR-1
- Deoxynucleotide (dNTP) Mix, 10 mM, Product No. D7295
- Lambda DNA EcoR I Hind III digest, Product No. D9281
- Gel Loading Solution , Product No. G2526
- EcoR I (40,000 units/ml), Product No. R4640
- TBE Buffer, 5X Concentrate, Product No. T6400
- Water, Molecular Biology, Product No. W4502

Related Books

Nucleic Acid Blotting: The Basics, Darling, D.C. *et al.*, Oxford University Press, Walton Street, Oxford, 1994 (Product No. Z35,763-4)

Current Protocols in Molecular Biology, Ausubel, F.M. *et al.* (John Wiley & Sons, NY, 1995)

†The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.