Genomic DNA Isolation Kit

Product No. **GDI-3**
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ProductInformation

TECHNICAL BULLETIN

Product Description

Sigma's Genomic DNA Isolation Kit isolates genomic DNA from small or large samples of fresh or frozen blood, suspension cells, trypsinized cells, mammalian tissues, *E. coli*, yeast, plant tissue and mouse tails. The procedures are relatively quick and simple. In general, the cells are lysed by the addition of Lysis Solution A followed by incubation at 65°C. The proteins and lipids are separated from the DNA by the addition of Precipitation Solution B and chloroform. Following centrifugation, the solution separates into two phases with a unique solid interface separating the two. The DNA is found in the upper aqueous phase, the proteins and lipids in the solid interface, while the chloroform forms the lower phase. The DNA is removed, ethanol precipitated, and resuspended in TE buffer. The resulting DNA is suitable for further manipulation, such as restriction digestion or PCR†. All samples yield high quality DNA up to 200 kb. The total process takes less than 90 minutes with fewer manipulations than other purification kits. A 30 minute procedure is included for isolating DNA from small volumes of blood for PCR or RFLP.

Reagents Provided

Sufficient for 15-200 isolations

- Lysis Solution A, Product No. L6283 — 55 ml
- DNA Precipitation Solution B, Product No. D6299 — 25 ml
- TE Buffer, Product No. T8178 — 100 ml
  10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8.0

Part A

- Mussel Glycogen, Product No. M0921 — 1 vial
  800 µl (2 mg/ml in sterile deionized water)
- RNase, Product No. R2892 — 1 vial
  800 µl (2 mg/ml in sterile deionized water)
- Protein Degrader, Product No. P5700 — 1 vial
  800 µl (20 mg/ml in sterile deionized water)

Reagents Required But Not Provided

(Sigma product numbers are given where appropriate)

- Phenol: Chloroform: Isoamyl Alcohol 25:24:1, Product No. P2069
- Chloroform, Product No. C2432
- 100% ethanol, Product No. E7023
- 80%(v/v) ethanol
- YPD Medium for yeast culture, Product No. Y1375 or see Appendix A, Recipes
- SCED solution (for yeast), see Appendix A, Recipes
- 20%(w/v) Polyethylene glycol in 1 M NaCl, see Appendix A, Recipes
- Phosphate buffered saline (PBS), Product No. P3813
- Water, molecular biology grade, Product No. W4502

Precautions and Disclaimer

Sigma's Genomic DNA Isolation Kit is for laboratory use only. Not for drug, household or other uses. Kit contains components that are hazardous. Warning statements are included on the labels.

Storage

The kit is composed of two boxes. Upon receipt store the smaller inner box (GDI-3 Part A) at -20°C and the larger outer box at room temperature as indicated on the labels.

Preparation Instructions

If Lysis Solution A precipitates, warm to 45°C and swirl to dissolve.

If Precipitation Solution B precipitates, stir the solution before use, allow the precipitate to settle and avoid precipitate when pipetting. Including a small amount of precipitate may improve DNA recovery in some extraction methods.

If Protein Degrader precipitates, warm to 37°C for 5 minutes.

Recipes for additional reagent solutions that may be required are given in Appendix A.

Procedures

Procedure #1 - Small Blood Samples and Hair Follicles
Procedure #2 - Small Amounts of Cells, Tissue, or Plant Leaves
Procedure #3 - Large Samples of Tissues, Cells, or Blood
Procedure #4 - Yeast Cells
Procedure #5 - Baculovirus
Procedure #6 - Mouse Tails

Note: All steps are performed at room temperature unless otherwise indicated.

Procedure #1 - Small Blood Samples and Hair Follicles

Before Starting:
Chill 100% and 80% ethanol solutions to -20°C.
Thaw mussel glycogen and keep on ice.
Equilibrate a heating block or water bath to 65°C

Sample Preparation:
Place 1 to 100 µl of fresh, frozen, or dried blood or hair follicle(s) in microcentrifuge tube(s). Blood should be a homogenous solution.

1. Add 50 µl of Lysis Solution A to the sample(s) and vortex in 1 second intervals until evenly dispersed.
2. Incubate at 65°C for 10 minutes.
3. Add 20 µl of Precipitation Solution B and vortex vigorously until the precipitate moves freely and the sample is uniformly viscous (10 seconds to 1 minute).
4. Add 70 µl of chloroform and vortex until viscosity decreases and the mixture is homogeneous (10 seconds to 1 minute).
5. Centrifuge at maximum speed (~14,000 X g) in a microcentrifuge for 10 minutes at 4°C to separate the phases and form the interface. Transfer the upper aqueous phase to a fresh microcentrifuge tube.
6. Add 445 µl TE buffer and 5 µl mussel glycogen to DNA solution. Add 1 ml of 100% ethanol (-20°C) and mix by inversion.
7. Incubate on ice for 30 minutes.
8. Centrifuge at maximum speed in a microcentrifuge for 10-15 minutes at 4°C. Remove ethanol with a drawn-out pipette while keeping the pellet intact.
9. Add 500 µl of 80% ethanol (-20°C) and mix by inverting the tube 3-5 times.
10. Centrifuge at maximum speed in a microcentrifuge for 3-5 minutes at 4°C. Remove the 80% ethanol with a drawn-out pipette.
11. Centrifuge at maximum speed in a microcentrifuge for 2-3 minutes at 4°C to remove any residual ethanol. Let the pellet air dry for 5 minutes.
12. Resuspend the pellet in 10 µl TE buffer. The purified DNA is ready for further experiments. Store at 2-8°C.

30 Minute Genomic DNA Extraction from Blood Samples.

Sample Preparation:
Pipette 350 µl of blood in a 2 ml microcentrifuge tube. Blood samples should be mixed until homogeneous.

1. Add 500 µl of Lysis Solution A to the sample and mix by inversion.
2. Incubate at 65°C for 6 minutes and mix by inversion.
3. Add 900 µl of chloroform and vortex vigorously.
4. Add 200 µl of Precipitation Solution B and vortex briefly until the sample is uniformly viscous.
5. Centrifuge at maximum speed (~14,000 X g) in a microcentrifuge for 5-10 minutes.
6. Transfer the clear aqueous phase to a new 2 ml microcentrifuge tube.
7. Add 1 ml of room temperature 100% ethanol and mix by inversion until a precipitate forms (usually seen within 30-60 seconds). If a precipitate is not seen, allow the tube to stand at room temperature for 10 minutes.

8. Centrifuge at maximum speed in a microcentrifuge for 5 minutes.

9. Remove the ethanol while keeping the pellet intact.

10. Add 1 ml of room temperature 70% ethanol.

11. Centrifuge at maximum speed in a microcentrifuge for 2 minutes. Remove the ethanol while keeping the pellet intact.

12. Centrifuge at maximum speed for 1 minute to remove any residual ethanol. Air dry the pellet for 5 minutes.

13. Add 100-150 µl of molecular biology grade water to each tube.

14. Incubate at 65°C for 5 minutes. The DNA is now ready for PCR or RFLP analysis.

Procedure #2 - Small Amounts of Cells, Tissue, or Plant Leaves

Sample Size:

10^3-10^7 suspension or trypsinized cells
0.5-1.0 ml *E. coli* cells of an overnight culture (~1X10^9 cells/ml)
3.5-100 mg of mammalian tissue
50 mg of fresh plant leaves

Before Starting:

Chill 100% and 80% ethanol solutions at -20°C.
Thaw RNase and keep on ice.
Equilibrate two heating blocks or water baths at 37°C and 65°C

Sample Preparation:

Cells must be pelleted and resuspended in 200 µl of 1X PBS. This will eliminate the formation of a salt pellet when precipitating DNA.
Tissue and leaves must be frozen in liquid N_2 and pulverized with a mortar and pestle. Samples are then placed in microcentrifuge tubes for processing.
Note: Fresh, minced leaves will yield DNA, however the yield and quality will not be as high as when the samples are first frozen in liquid N_2.

1. Add 350 µl of Lysis Solution A to the sample and vortex in 1 second intervals until evenly dispersed.

2. Incubate at 65°C for 10 minutes

3. Add 150 µl of Precipitation Solution B and vortex vigorously until the precipitate moves freely and the sample is uniformly viscous (10 seconds to 1 minute).

4. Add 500 µl of chloroform and vortex until viscosity decreases and the mixture is homogeneous (10 seconds to 1 minute).

5. Centrifuge at maximum speed (~14,000 X g) for 10-20 minutes at 4°C to separate phases and form the interface. Transfer the upper phase into a fresh microcentrifuge tube.

6. Add 1 ml of 100% ethanol (-20°C) to the DNA solution and vortex briefly.

7. Incubate tube on ice for 30 minutes.

8. Centrifuge at maximum speed in a microcentrifuge for 10-15 minutes at 4°C. Remove ethanol from the pellet.

9. Add 500 µl of 80% ethanol (-20°C) and mix by inverting the tube 3-5 times.

10. Centrifuge at maximum speed for 3-5 minutes at 4°C. Remove the 80% ethanol while keeping the pellet intact.

11. Centrifuge at maximum speed for 2-3 minutes at 4°C to remove any residual ethanol. Let the pellet air dry for 5 minutes.

12. Resuspend the pellet in 100 µl TE buffer. Add 2 µl of a 2 mg/ml RNase solution, to bring the concentration to 40 µg/ml.

13. Incubate at 37°C for 30 minutes. The purified DNA is ready for further experiments.

Procedure #3 - Large Samples of Tissue, Cells or Blood

Sample Size:
10^7-10^8 suspension or trypsinized cells
100 mg to 1 g of mammalian tissue
100 µl to 2 ml of blood

Before Starting:
Chill 100% and 80% ethanol solutions at -20°C.
Thaw RNase and keep on ice.
Equilibrate two heating blocks or water baths at 65°C and 37°C

Sample Preparation:
Pellet cells and decant medium. It is not necessary to resuspend in 1X PBS.
Mammalian tissue must be frozen in liquid N_2 and pulverized with a mortar and pestle.
All samples should be placed into sterile 15 ml snap-cap polypropylene tubes.

1. Add 3.5 ml of Lysis Solution A to the sample and vortex in 1 second intervals until even dispersed.

2. Incubate at 65°C for 10 minutes.

3. Add 1.5 ml of Precipitation Solution B and vortex vigorously until the precipitate moves freely in the tube and the sample is uniformly viscous (10 seconds to 1 minute).

4. Add 5 ml of chloroform and vortex until the viscosity decreases and the mixture is homogeneous (10 seconds to 1 minute).

5. Centrifuge at 8,000 X g for 20 minutes at 4°C to separate the phases and form an interface. Carefully decant the upper aqueous phase into a fresh 15 ml tube.

6. Add 5 ml phenol: chloroform: isoamyl alcohol, (25:24:1) to the aqueous phase and vortex.

7. Centrifuge at 8,000 X g for 5 minutes at 4°C to separate the phases. Carefully decant the upper aqueous phase into a fresh 15 ml tube.

8. Add 10 ml of 100% ethanol (-20°C) to the DNA solution and vortex briefly.

9. Incubate the tube on ice for 30 minutes.

10. Centrifuge at 8,000 X g for 10-15 minutes at 4°C. Carefully decant the ethanol while keeping the pellet intact.

11. Add 5 ml of 80% ethanol (-20°C) and invert the tube 3-5 times.

12. Centrifuge at 8,000 X g for 3-5 minutes at 4°C. Remove the ethanol while keeping the pellet intact.

13. Centrifuge at 8,000 X g for 2-3 minutes at 4°C to remove any residual ethanol. Let the pellet air dry for 5 minutes.

14. Resuspend the pellet in 1 ml TE buffer. Add 20 µl of a 2 mg/ml RNase solution to bring the final concentration to 40 µg/ml.

15. Incubate at 37°C for 30 minutes. The purified DNA is ready for further experiments. Store at 2-8°C.

Procedure #4 - Yeast Cells

Before Starting:
Prepare SCED solution (see Appendix A, Recipes).
Chill 100% and 80% ethanol solutions at -20°C.
Thaw RNase and keep on ice.
Equilibrate two heating blocks or water baths at 65°C and 37°C

Sample Preparation:
Prepare YPD medium (see Appendix A, Recipes).
Inoculate 10 ml of YPD medium with a single yeast colony and grow overnight. This will be the sample source.

1. Transfer 10 ml of the overnight culture to a 15 ml snap-cap polypropylene tube. Centrifuge the cells at 4,000 X g for 5-10 minutes at 4°C. Carefully decant the medium without disturbing the DNA pellet.

2. Add 10 ml of sterile, deionized water and tap the tube to resuspend the cells. Centrifuge the cells at 4,000 x g for 5-10 minutes at 4°C. Decant the water.

3. Resuspend the pellet in 2 ml SCED and incubate at 37°C for 1 hour.

4. Add 3.5 ml of Lysis Solution A to yeast cells and vortex in 1 second intervals until evenly dispersed.

5. Incubate at 65°C for 10 minutes.

6. Add 1.5 ml of Precipitation Solution B and vortex vigorously until the precipitate moves freely in the tube and the sample is uniformly viscous (10 seconds to 1 minute).

7. Add 5 ml of chloroform and vortex until the viscosity decreases and the mixture is homogeneous (10 seconds to 1 minute).

8. Centrifuge at 1,000 X g for 20 minutes at 4°C to separate the phases and form an interface. Carefully decant the upper, aqueous phase in a fresh 15 ml tube.

9. Add 10 ml of 100% ethanol (-20°C) to the DNA solution, vortex briefly, and incubate the tube on ice for 30 minutes.

10. Centrifuge at 4,000 X g for 10-15 minutes at 4°C. Carefully decant the ethanol while keeping the pellet intact.

11. Add 5 ml of 80% ethanol (-20°C) and invert the tube several times to wash the pellet. Centrifuge at 4,000 X g for 3-5 minutes at 4°C.

12. Decant the ethanol, centrifuge at 4,000 X g for 2-3 minutes at 4°C, and remove any residual ethanol with a pipette. Let the pellet air dry for 5 minutes.

13. Resuspend the pellet in 100 µl TE buffer. Add 2 µl of a 2 mg/ml RNase solution for a final concentration of 40 µg/ml. Incubate the sample at 37°C for 30 minutes.

14. Centrifuge the tube at 4,000 X g for 1 minute at 4°C to remove insoluble particles.

15. Transfer the supernatant to a fresh microcentrifuge tube. The purified DNA is ready for further experiments. Store at 2-8°C.

Procedure #5 - Baculovirus

Before Starting:

Prepare 20% PEG in 1 M NaCl and chill at 4°C (see Appendix A, Recipes).

Chill 100% and 70% ethanol solutions at -20°C.

Equilibrate a heating block or water bath at 65°C

Sample Preparation:

From a 6- or 12-well microtiter plate choose an occlusion-negative well and disrupt the cell monolayer with a sterile pipette tip.

1. Transfer 750 µl of an occlusion-negative cell suspension to a microcentrifuge tube. Centrifuge at 5,000 rpm in a microcentrifuge for 3 minutes to pellet the cells. Transfer the supernatant to a fresh microcentrifuge tube.

2. Add 750 µl of cold (4°C) 20% PEG in 1 M NaCl to the tube. Mix three times by inversion and incubate on ice for 30 minutes.

3. Centrifuge at maximum speed (~14,000 X g) for 10 minutes at 4°C to pellet the viral particles. Keep the pellet and discard the supernatant.

4. Centrifuge the tube again at maximum speed for 2 minutes at 4°C. Remove the residual supernatant with a pipette.

5. Resuspend the viral particles in 100 µl of TE buffer.

6. Add 143 µl Lysis Solution A to the resuspended viral particles and vortex 1 second to mix.

7. Incubate the sample at 65°C for 6 minutes.

8. Add 58 µl of Precipitation Solution B and vortex vigorously for 5 seconds until the mixture is uniform and there is no white plug in the bottom of the tube.

9. Add 258 µl of chloroform and vortex until evenly mixed.

10. Centrifuge at maximum speed for 10 minutes at 4°C to separate the phases and create an interface. Transfer the upper, aqueous phase to a fresh microcentrifuge tube.

11. Add 500 µl of 100% ethanol (-20°C) to the DNA solution. Invert the tube eight times to precipitate the DNA.

12. Centrifuge at maximum speed for 5 minutes at 4°C. Decant the ethanol while keeping the pellet intact.

13. Add 500 µl of 70% ethanol (-20°C) and centrifuge at maximum speed for 5 minutes at 4°C. Decant the ethanol while keeping the pellet intact.

14. Centrifuge at maximum speed for 2-3 minutes at 4°C to remove any residual ethanol. Let the pellet air dry for 5 minutes.

15. Resuspend the pellet in 20 µl of TE buffer. The purified DNA is ready for further experiments. Store at 2-8°C.

Procedure #6 - Mouse Tails

Day 1 - Before Starting:
Thaw protein degrader and keep on ice.
Equilibrate a shaker water bath at 60°C

1. To a fresh microcentrifuge tube, mix:

320 µl	TE
20 µl	Lysis Solution A
10 µl	Precipitation Solution B
5 µl	Protein Degrader

2. Add 1 cm of freshly cut mouse tail to the microcentrifuge tube and shake (225 rpm) the tube on its side at 60°C overnight (12-24 hours).
Note: After incubation, the mouse tail should be totally digested with only tiny pieces of bone in the bottom of the tube. The solution will be cloudy and may be slightly colored depending on the color of the mouse tail.

Day 2 - Before Starting:
Chill 100% and 80% ethanol solutions at -20°C.
Thaw RNase and keep on ice.
Equilibrate a heating block or water bath at 37°C

3. Add 300 µl of Lysis Solution A and 120 µl of Precipitation Solution B to the sample and vortex vigorously until the solution is uniformly viscous (10 seconds to 1 minute).

4. Add 750 µl of chloroform and vortex until the mixture is homogeneous (10 seconds to 1 minute).

5. Centrifuge at maximum speed (~14,000 X g) for 10 minutes at 4°C and transfer upper aqueous phase to a fresh microcentrifuge tube.

6. If the upper phase is not clear, a second chloroform extraction is needed. Repeat steps 4 and 5. When the upper phase is clear, proceed to step 7.

7. Add 1.0 ml of 100% ethanol (-20°C) to the upper phase. Vortex the sample and incubate on ice for 30 minutes.

8. Centrifuge at maximum speed for 10-15 minutes at 4°C. Remove the ethanol leaving the pellet intact.

9. Add 500 µl of 80% ethanol (-20°C) and mix by inverting the tube 3-5 times.

10. Centrifuge at maximum speed for 3-5 minutes at 4°C. Remove the ethanol while leaving the pellet intact.

11. Centrifuge the tube at maximum speed for 1-3 minutes at 4°C to remove any residual ethanol. Let the pellet air dry for 5 minutes.

12. Resuspend the pellet in 49 µl of TE and add 1 µl of a 2 mg/ml solution of RNase to a final concentration of 40 µg/ml. Incubate the sample at 37°C for 30 minutes. The DNA is now ready for further experiments. Store at 2-8°C.

Guidelines for Procedure Selection
When none of the procedures match the type or size of sample used, the following table offers suggestions for procedure selection.

Sample size	Suggested procedure
Small or valuable (1 to 100 µl or <1 mg)	Procedure #1
Moderate (3 to 100 mg tissue, 10^3-10^7 mammalian cells or 10^9 bacterial cells)	Procedure #2
Large (0.1 to 1.0 g tissue or 10^7-10^8 mammalian cells)	Procedure #3
Other yeast cells	Procedure #4
Viral particles	Procedure #5

Use of RNase

If the sample size is small consisting of terminally differentiated cells, RNase is not required.
If the sample size is moderate consisting of viral particles, RNase is not required.
If the sample size is moderate or large consisting of terminally differentiated cells or tissue, bacterial cells, yeast cells, or tissue culture cells, add 40 µg/ml RNase.

Use of Protein Degrader

If the sample size is small consisting of terminally differentiated cells, protein degrader is not required.
If the sample size is moderate consisting of tissue with high amounts of protein, i.e. connective tissue, use 100 µg of protein degrader and follow Procedure #6.
If the sample size is large consisting of tissue with high amounts of protein, i.e. connective tissue, use 1.0 mg of protein degrader and scale-up Procedure #6.

Use of Mussel Glycogen

If the sample is small or the DNA is very dilute, then use mussel glycogen at a final concentration of 20 µg/ml to precipitate DNA.

Results

Expected Yields

Sample	Expected Yield
10 µl of Blood	20-30 ng
10^7 Sf9 cells	~170-180 µg
10^9 *E. coli* cells	~30-40 µg
50 mg leaf tissue	~4 µg
50 mg of human liver tissue	~150 µg
87 mg of human brain tissue	~170 µg
500 mg of rat heart tissue	~100 µg
2 ml of blood	~40 µg
4 x 10^9 *Pichia pastoris* cells	~850 µg
Mouse tail (1 cm)	~125 µg
1 hair follicle	~5-10 ng

Troubleshooting Guide

Problem	**Solution**
No interface	Confirm using Lysis Solution A and Precipitation Solution B correctly.
No phase	Confirm that chloroform was added.
A colored interface	Interface often picks up the pigments from sample. Not a problem.
Low yield or No DNA	Use mussel glycogen to precipitate the DNA.
	Add 100 µl of Protein Degrader and follow Procedure #6.
	Freeze sample in liquid nitrogen and pulverize with a mortar and pestle.
	Include small amount of the precipitate that is in Precipitation Solution B.
Degraded DNA	Add 100 µl of Protein Degrader and follow Procedure #6.
	Be sure all solutions are not contaminated
RNA in the sample	Add 40 µg/ml RNase and incubate for 30 minutes at 37°C.
A large salt pellet after precipitating the DNA	Resuspend the pellet in 50 µl TE buffer and add 50 µl of 4 M ammonium acetate. Add 200 µl of 100% ethanol (-20°C) to precipitate the DNA. Centrifuge to pellet the DNA and wash with 80% ethanol. Air dry the pellet for 5 minutes. Resuspend the pellet in the desired volume of TE buffer.

Appendix A
Reagent Recipes

YPD Medium
1% Yeast extract
2% Peptone
2% Glucose

1. Dissolve 10 g of Yeast extract (Product No. Y1625) and 20 g of Peptone (Product No. P0556) in 960 ml of deionized water.

2. Autoclave at 15 lbs/sq. in. for 20 minutes.

3. Make a 50% glucose solution by dissolving 50 g of glucose (Product No. G5400) in 100 ml of deionized water. Filter sterilize.

4. When the autoclaved solution has cooled, add 40 ml of the 50% glucose.

SCED Solution
1 M Sorbitol
1 mM EDTA
10 mM Sodium Citrate, pH 5.8
10 mM Dithiothreitol (DTT)

1. For 1 L of SCE, dissolve 182.2 g of sorbitol (Product No. S6021), 0.372 g EDTA (Product No. E5134) and 2.94 g sodium citrate (Product No. C8532) in 900 ml deionized water. Adjust the pH to 5.8 with HCl.

2. Bring the volume up to 1 L and autoclave at 15 lbs/sq. in. for 20 minutes. Store at room temperature.

3. For 10 ml of SCED, make 10 ml of a 1 M DTT solution by dissolving 1.54 g of DTT (Product No. D9779) in 10 ml of water. Aliquot the 1 M DTT into 1 ml samples and store at -20°C. Stable for at least 6 months.

4. Add 100 µl of the 1 M DTT solution to 10 ml of SCE. Use immediately.

20% PEG in 1 M NaCl

1. For 100 ml, mix 20 g PEG 8000 (Product No. P5413) and 5.84 g of NaCl (Product No. S3014) in 100 ml of water.

2. Autoclave 20 minutes at 15 lbs/sq. in.

3. While the solution is still warm, gently swirl to mix thoroughly