# **Purification**

#### **GENOMIC DNA PURIFICATION**

#### Extract-N-Amp™ Blood PCR Kits

#### From whole blood to PCR in under 8 minutes.

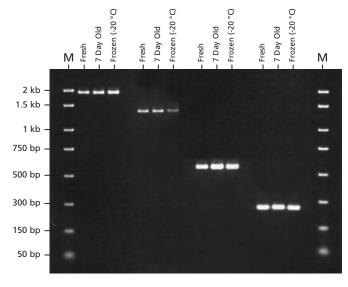
The Extract-N-Amp™ Blood PCR Kits contain all of the reagents necessary to rapidly extract host genomic DNA from whole blood and amplify targets of interest by PCR (Fig. 1). This novel extraction system eliminates the need for any type of purification, organic extraction, centrifugation, heating, filtration or alcohol precipitation. The kit also includes a PCR Ready mix, especially formulated for amplification directly from the extract. This formulation uses an antibody based Hot Start, for specific amplification. The PCR master mix comes in two formulations: Extract-N-Amp™ Blood PCR mix and REDExtract-N-Amp™ Blood PCR mix. The REDExtract-N-Amp™ Blood PCR kit contains a tracking dye that allows for convenient direct loading of PCR reactions onto agarose gels for analysis.

Genomic DNA is extracted from 10 µl of whole blood by simply adding the Extraction Solution and incubating for 5 minutes at room temperature. The Neutralization Solution is added to the extract to counteract inhibitory substances prior to PCR. A portion of the DNA extract is then added to the specially formulated PCR mix.

#### **Features and Benefits**

- Efficient 8 minute prep allows greater speed and throughput
- No need for any type of purification, organic extraction, centrifugation or alcohol precipitation
- Simple, 3 step procedure with no special equipment required
- Hot Start antibody included for highly specific PCR amplification of genomic DNA
- Compatible with multiple format (single tube, or 96-well)
- Can be used with whole blood or blood cards
- Extract stable at 4 °C for at least 6 months (Fig. 2)

Storage: -20-0 °C Shipped in wet ice R: 34 S: 26-27-36/37/39



#### Overview of Extract-N-Amp™ **Blood PCR Kit Procedure**



Incubate whole blood sample with extraction solution for 5 min at room temperature



Add neutralization solution



Mix aliquot with primers and REDExtract-N-Amp™ or Extract-N-Amp™ PCR ReadyMix



Transfer to thermal cycler



Directly load amplified PCR product on gel



PCR analysis of genomic DNA isolated from blood using Sigma's Extract-N-Amp™ Blood PCR Kit.

Figure 1. Extract-N-Amp™ Blood PCR Kit used to isolate genomic DNA from fresh, 7 day old, & frozen blood. DNA was extracted and neutralized from 10 µl of whole blood in 5 minutes at room temperature using the REDExtract-N-Amp™ Blood PCR kit. The PCR products were then generated using the specially formulated Hot Start PCR mix included in the kit. PCR products generated are 1.8 kb for carnitine palmitoyltransferase II, 1.3 kb for a mitochondrial DNA control region, 547 bp for human surfactant protein B, and 320 bp for the 5' untranslated region of human major histocompatibility complex class II.

#### Stability of Blood Extracts at 37 °C

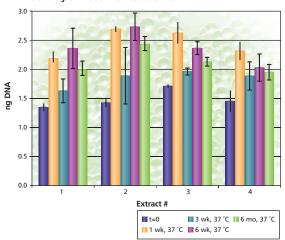


Figure 2. Stability of Extract-N-Amp<sup>TM</sup> Blood Extracts. Blood was drawn from 2 human volunteers into vacutainer tubes containing EDTA. Extractions were performed in duplicate providing 4 samples total. Half the extracts were stored at 4 °C (recommended storage conditions) and the other half at 37 °C (accelerated storage). Samples were removed at various time intervals for testing. Stability was determined by monitoring yield from quantitative PCR using an ABI 7700 instrument. The DNA standards used for the quantitative PCR were generated from the same blood draw as the test samples, purified using the GenElute Blood Genomic DNA Kit (NA2000) and stored as single aliquots at -20 °C. The PCR products were generated using primers for a 547 bp product from human surfactant protein B (SPB; Lin & Floros, 2000, BioTechniques, 29: 460-466). The results clearly show no loss of amplification of the SPB PCR product even after storage at 37 °C for 6 months. Similar results were obtained with storage at 4 °C.

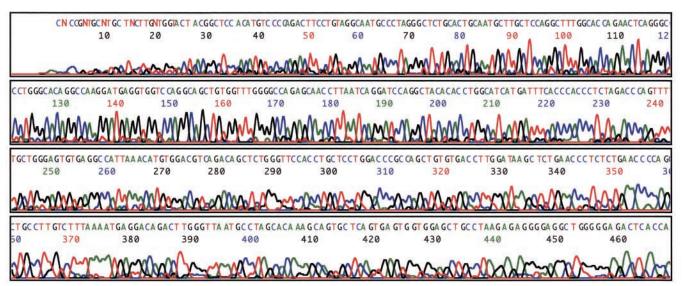


Figure 3. Direct sequence from PCR products generated using the Extract-N-Amp™ Blood Kit. A 547 bp product for human surfactant protein B was generated using the Extract-N-Amp Blood PCR kit. The product was sequenced directly using BigDye™ terminator chemistry. Sequencing reactions were resolved on an ABI 3100.

Note: Some PCR products require further clean-up prior to sequencing. The GenElute™ PCR Clean-Up Kit (NA1020) is recommended.

Product	Product Description	Extractions	Amplifications
<u>XNABS</u>	REDExtract-N-Amp™ Blood PCR Kit (contains REDTaq)	10	10
<u>XNAB</u>	REDExtract-N-Amp™ Blood PCR Kit (contains REDTaq)	100	100
<u>XNABE</u>	REDExtract-N-Amp™ Blood PCR Kit (contains REDTaq)	100	500
<u>XNABR</u>	REDExtract-N-Amp™ Blood PCR Kit (contains REDTaq)	1000	1000
<u>XNABRE</u>	REDExtract-N-Amp™ Blood PCR Kit (contains REDTaq)	1000	5000
XNAB2	Extract-N-Amp™ Blood PCR Kits	100	100
XNAB2E	Extract-N-Amp™ Blood PCR Kits	100	500
XNAB2R	Extract-N-Amp™ Blood PCR Kits	1000	1000
XNAB2RE	Extract-N-Amp™ Blood PCR Kits	1000	5000

#### **Extract-N-Amp™ Plant PCR Kits**

#### From leaf tissue to PCR in under 15 minutes.

The Extract-N-Amp™ Plant PCR Kits contain all the reagents necessary to rapidly extract genomic DNA from plant leaves and amplify targets of interest by PCR (Fig. 1). A novel Extraction Solution eliminates the need for conventional freezing of plant tissues with liquid nitrogen, mechanical disruption, organic extraction, column purification, or precipitation of DNA. The kit also includes a PCR reaction mix, especially formulated for amplification directly from extract. This formulation uses an antibody based Hot Start for specific amplification. The PCR master mix comes in two formulations: Extract-N-Amp™ PCR Reaction Mix and REDExtract-N-Amp™ Plant PCR Kit. The REDExtract-N-Amp™ PCR mix contains a dye that acts as a tracking dye and allows for convenient direct loading of PCR reactions onto agarose gels for analysis.

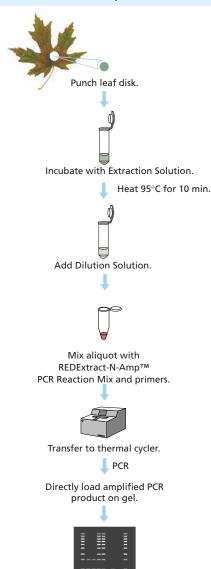
Genomic DNA is extracted from 0.5 to 0.7 cm plant leaf disks that have been cut with a standard paper punch and simply incubated in Extraction Solution at 95 °C for 10 minutes. An equal volume of Dilution Solution is added to the extract to neutralize inhibitory substances prior to PCR. A portion of the DNA extract is then added to a PCR reaction containing primers and either the REDExtract-N-Amp<sup>TM</sup> or Extract-N-Amp<sup>TM</sup> PCR.

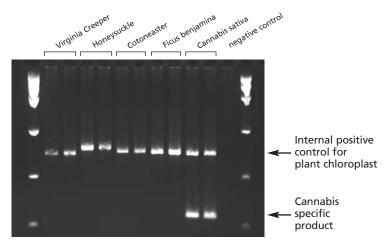
#### **Features and Benefits**

- Single-step extraction of plant genomic DNA for PCR in less than 15 minutes
- No freezing, mechanical disruption, organic extraction, column purification or precipitation required
- Specially formulated PCR Ready Mix for use with extract
- Hot Start antibody for highly specific PCR amplification of genomic DNA
- REDExtract-N-Amp™ requires no loading buffers or tracking dyes required for gel analysis
- Compatible with high-throughput requirements for genetic analysis of plants
- Extract stable at 4 °C for at least 6 months (Fig. 3)

**Storage:** -20-0 °C Shipped in wet ice R: 36/37/38 S: 26-36

#### Overview of Extract-N-Amp™ Plant PCR kit procedure.





PCR analyses of genomic DNA extracted from 5 different plant species using Sigma's Extract-N-Amp™ Plant Kit.

Figure 1. Extract-N-Amp™ Plant PCR Kit used to isolate and amplify genomic DNA from various plant sources. Genomic DNA was extracted from 0.5 cm leaf disks that were cut using a standard paper punch. DNA was extracted using the Extract-N-Amp™ Plant PCR Kit in less than 15 minutes. All samples were then amplified using the specially formulated Hot Start PCR mix. The products were generated from a 30-cycle duplex reaction containing primers specific to plant chloroplast (upper band) and primers specific to Cannabis sativa DNA (lower band). MW ladder is 100, 200, 400 and 800 bp. Data provided by Andy Hopwood, Forensic Science Service, Birmingham, England.

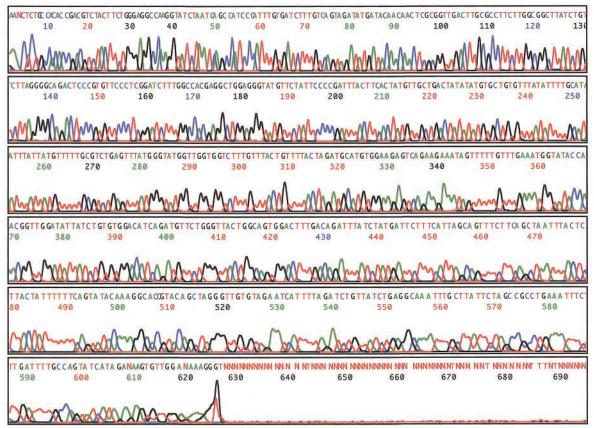
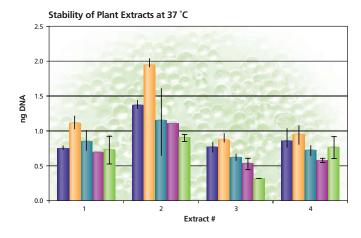


Figure 2. Sequence was resolved on a ABI 310 from a purified, 645 bp corn leaf PCR product. The PCR product was purified with the GenElute™ PCR Clean-Up Kit. The DNA extraction and PCR were performed using Sigma's Extract-N-Amp™ Plant PCR kit. The sequence was obtained by using ABI BigDye™ Terminator Chemistry and the same primers as for the original PCR.



### ■ t=0 ■ 3 wk, 37 °C ■ 6 mo, 37 °C ■ 1 wk, 37 °C ■ 6 wk, 37 °C

#### Stability of DNA in corn leaf extracts.

Figure 3. Eight disks were punched from a corn leaf, and DNA was extracted according to the procedure in the Technical Bulletin for the Extract-N-AMP Plant Kit. Two 4-µl aliquots from each were analyzed immediately by quantitative PCR with SYBR® Green detection on an ABI Prism 7700. DNA standards for quantitative PCR were purified DNA prepared from corn leaf tissue with the GenElute Plant Genomic DNA kit (Product Code G2N70). Half of the leaf extracts were stored at 4 °C (recommended storage conditions) and the other half at 37 °C (accelerated storage). Quantitative PCR was repeated after 1, 3, and 6 months from extracts at 4 °C, and after 1 week, 3 weeks, 6 weeks, and 6 months from extracts at 37 °C. Results for storage at 37 °C are shown. The average of 2 replicate PCR assays from each extract is plotted. Error bars represent one standard deviation. Results for storage at 4 °C are essentially the same as those shown for 37 °C.

Product	Product Description	Extractions	Amplifications
<u>XNAPS</u>	REDExtract-N-Amp™ Plant	10	10
XNAP	REDExtract-N-Amp™ Plant	100	100
<u>XNAPE</u>	REDExtract-N-Amp™ Plant	100	500
<u>XNAPR</u>	REDExtract-N-Amp™ Plant	1000	1000
<u>XNAPRE</u>	REDExtract-N-Amp™ Plant	1000	5000
XNAP2	Extract-N-Amp™ Plant	100	100
XNAP2E	Extract-N-Amp™ Plant	100	500
XNAR	Extract-N-Amp™ Plant	1000	1000
XNAP2RE	Extract-N-Amp™ Plant	1000	5000



#### **GenElute™ Mammalian Genomic DNA Miniprep Kits**

For purification of genomic DNA from a variety of mammalian sources.

The GenElute™ Mammalian Genomic DNA Purification Kit provides a simple and convenient way to isolate pure, high molecular weight DNA from a variety of mammalian sources (Figs. 1 and 2). These kits use a silica-based membrane, specially selected for genomic DNA purification, in a convenient spin column format. Mammalian cells and tissues are lysed with a chaotropic salt-containing buffer to ensure denaturation of macromolecules. DNA is bound to the spin column membrane and the remaining lysate is removed by centrifugation. A filtration column is used to remove cell debris, after washing to remove contaminants; the DNA is eluted with buffer into a collection tube. The purified DNA may be used in many applications such as sequencing, cloning, blotting, restriction digestion (Fig. 3), ligation, and PCR.

#### **Features and Benefits**

- Typical DNA yields of 25  $\mu g$  from 2 x 10 $^{6}$  cultured cells or 30  $\mu g$ from 25 mg of tissue (Fig. 4)
- Preparation time is only 20 minutes after lysis
- Purified genomic DNA has A<sub>260</sub>/A<sub>280</sub> ratios between 1.6 and 1.9
- No need for mechanical homogenization
- 40% more purification preps offered than market leader

**Storage:** Room Temperature R: 20/21/22 S: 26-36

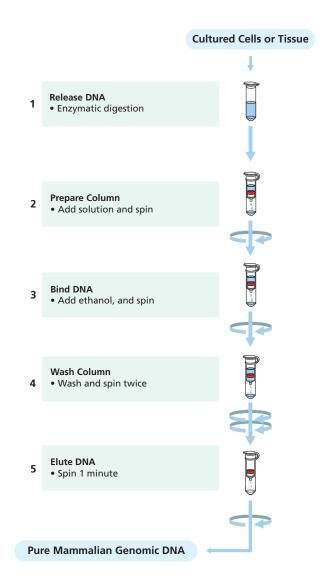
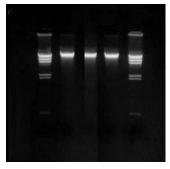


Table 1. Yields produced using GenElute™ Mammalian Genomic DNA Purification Kits\*

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Material	Amount	Typical Yield
Jurkat Cells (human)	2 x 10 <sup>6</sup> Cells	5-10 μg
HEK293 Cells (human)	2 x 10 <sup>6</sup> Cells	10-20 μg
HeLa Cells (human)	2 x 10 <sup>6</sup> Cells	15-25 μg
Mouse Pancreas Tissue	20 mg	10-25 μg
Mouse Spleen Tissue	10 mg	10-25 μg
Mouse Thymus Tissue	16 mg	10-25 μg
Mouse Lung Tissue	20 mg	5-15 μg
Mouse Brain Tissue	16 mg	5-15 μg
Mouse Kidney Tissue	20 mg	10-25 μg
Mouse Liver Tissue	25 mg	10-30 μg

<sup>\*</sup>With RNase and Proteinase K treatment

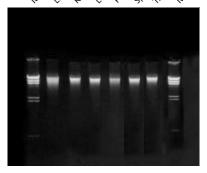
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Genomic DNA purified from cells using GenElute™ Mammalian Genomic DNA Purification Kit.

Figure 1. Genomic DNA purified from cells. Purified genomic DNA was isolated with the GenElute™ Mammalian Genomic DNA Purification Kit from 2 x 10<sup>6</sup> cells of the sources indicated. The genomic DNA (200 ng/lane) was analyzed on a 0.8% agarose gel. Markers are lambda DNA digested with Hind III.

### Varied Lind Lung 20 mg



Genomic DNA purified from tissues using GenElute™ Mammalian Genomic DNA Purification Kit.

Figure 2. Genomic DNA purified from mammalian tissues. Purified genomic DNA, from the indicated mouse tissues, was isolated with the GenElute™ Mammalian Genomic DNA Purification Kit. Genomic DNA (200 ng/lane) was analyzed on a 0.8% agarose gel to illustrate yield and integrity. Markers are lambda DNA digested with Hind III.

## HeLa HEK Liver the Uncur Cut Uncut Marke

Genomic DNA purified with GenElute™ Mammalian Genomic DNA Purification Kit is suitable for restriction enzyme digestions. Figure 3. Genomic DNA (200 ng) was digested with EcoR I (10 units in 20 µI at 37 °C for 4 hours) followed by electrophoresis (150 ng/lane) on a 0.8 % agarose gel. The undigested DNA was incubated under the same conditions, but without EcoR I. Markers are lambda DNA digested with Hind III.

#### **Consistently Higher Yield Than the Market Leader**

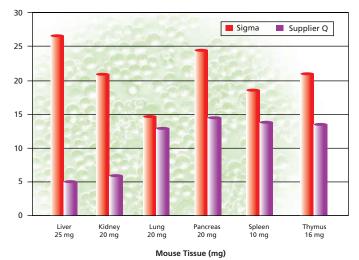


Figure 4. Yields of genomic DNA compared with leading Supplier Q using 2 x 200 μl elutions.

Product	Product Description	Preps	Quantity
<u>G1N10</u>	GenElute™ Mammalian Genomic DNA Miniprep	10	1 kit
<u>G1N70</u>	GenElute™ Mammalian Genomic DNA Miniprep	70	1 kit
G1N350	GenElute™ Mammalian Genomic DNA Miniprep	350	1 kit



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#### **GENOMIC DNA PURIFICATION**

#### **GenElute™ Plant Genomic DNA Miniprep Kits**

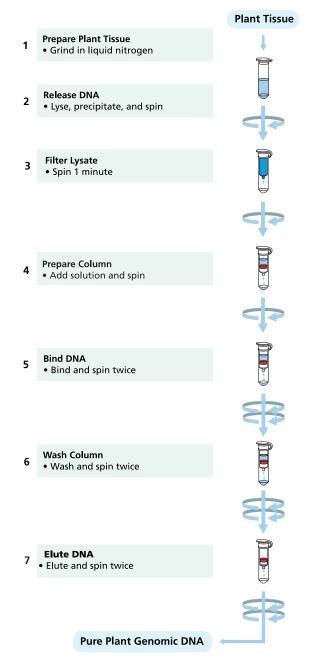
For Purification genomic DNA from a variety of plant species.

With the GenElute™ Plant Genomic DNA Miniprep Kit, high quality genomic DNA can be purified from a variety of plant species (Table 1, Fig. 1 and 2). The kit contains all the reagents, columns and tubes necessary to isolate genomic DNA from up to 100 mg of fresh or 20 mg of freeze-dried plant tissue. Plant tissue is disrupted by grinding in liquid nitrogen, and DNA is released with detergent and chaotrope. Proteins, polysaccharides, and cell debris are eliminated with a 10 minute precipitation procedure followed by centrifugation through a filtration column, included in the kit. The genomic DNA is purified further by a silica bind-wash-elute procedure in microcentrifuge spin columns. Purified DNA is ready for downstream applications such as PCR (Fig. 3), restriction endonuclease digestions, cloning and southern blots.

#### **Features and Benefits**

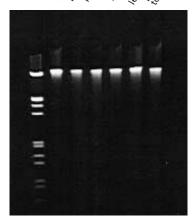
- Less than 40 minutes from tissue to purified genomic DNA, including disruption in liquid nitrogen (Fig. 4)
- Typical DNA yields of up to 20 μg per prep
- No RNase treatment required
- 40% more purifications per kit than leading supplier

**Storage:** Room Temperature R: 20/21/22 S: 26-36



#### Table 1. Typical yields of genomic DNA isolated from various plant species per 100 mg of starting leaf tissue.

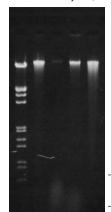
Material	Typical Yield
Corn	7.5 µg
Dianthus tissue culture	3.3 µg
Pepper	3.1 μg
Rice	5.9 μg
Soybean	5.7 μg
Tobacco	5.2 μg
Tomato	6.2 μg
Tomato (20 mg freeze dried leaf tissue)	5.7 μg
Wheat	11.5 μg



Genomic DNA from various plant species isolated with GenElute™ Plant Genomic DNA Miniprep Kit.

Figure 1. Purified genomic DNA (0.4 µg/lane) was analyzed on a 0.8% agarose gel. Markers are Lambda Hind III digest.

## SupplierM



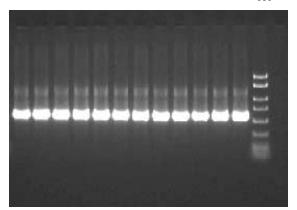
Genomic DNA isolated from 100 mg fresh tomato leaves using various

Figure 2. Purified genomic DNA (0.4  $\mu g/lane$ ) was analyzed on a 0.8% agarose gel. Sigma, membrane-based; Supplier A, resin-based; Supplier M, solution based; Supplier Q, membrane-based.

Note: RNA contamination present in DNA isolated using the kits from both Supplier A and M.

RNA

#### M



#### PCR amplification of a 500 bp product isolated from genomic DNA.

Figure 3. Genomic DNA from soybean leaves was purified using the GenElute™ Plant Genomic DNA Miniprep Kit. A 5 µl aliquot of eluate was used as template in a 20  $\mu$ l total PCR reaction for 30 cycles. A 5  $\mu$ l aliquot of each PCR reaction was resolved on a 2% precast agarose gel (Product Code <u>P 5722</u>). The PCR marker (M) used (Product Code <u>P 9577</u>) ranged from 50 bp to 2 kb.

#### **Supplier Comparison of Preparation Time**

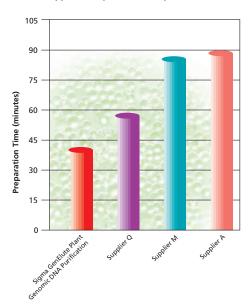


Figure 4. Comparison of preparation time. The prep time required using the GenElute™ Plant Genomic DNA Miniprep Kit, compared to that of three kits from other suppliers.

#### **ORDERING INFORMATION**

Product	Product Description	Preps	Quantity
<u>G2N10</u>	GenElute™ Plant Genomic DNA Miniprep	10	1 kit
<u>G2N70</u>	GenElute™ Plant Genomic DNA Miniprep	70	1 kit
G2N350	GenElute™ Plant Genomic DNA Miniprep	350	1 kit

Order: 1.800.325.3010

5.583

1.800.32

Service:

Technical

#### GenElute™ Blood Genomic DNA Kits

For purification of genomic DNA from fresh or aged whole blood.

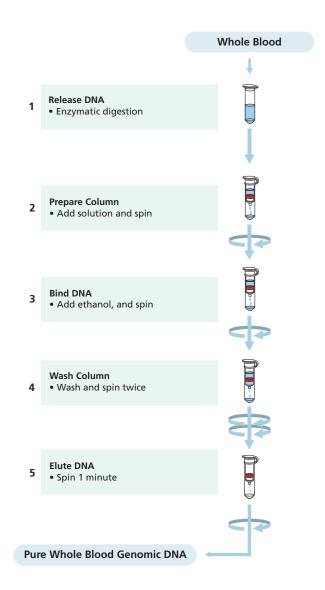
Sigma's GenElute™ Blood Genomic DNA Kit provides a simple and convenient technique to isolate high quality genomic DNA from fresh or aged (older than 24 hours) whole blood. This kit combines the advantages of a silica-based system with a microspin format, eliminating the need for expensive resins and hazardous organic compounds. Whole blood is digested w/Proteinase K and is lysed following the addition of a chaotropic salt-containing solution. DNA is bound to the silica-based membrane and the remaining lysate is removed by centrifugation. After washing, to remove contaminants that are associated with aged whole blood samples, the DNA is eluted with buffer into a collection tube. Purified DNA is ready for downstream applications such as restriction endonuclease digestions (Fig. 1), PCR (Fig. 3), Southern blots and sequencing reactions.

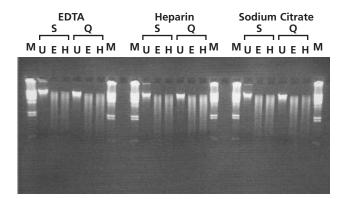
#### **Features and Benefits**

- Isolate high quality genomic DNA from fresh or aged blood
- Compatible with many anticoagulants, including EDTA, Heparin and Sodium Citrate (Figs. 2A and 2B)
- Less than 40 minutes from whole blood to isolated genomic DNA
- A prewash solution is provided to ensure the removal of all contaminants associated with older blood samples
- Purity of genomic DNA has an A<sub>260</sub>/A<sub>280</sub> ratio between 1.6 and 1.9
- An RNase solution is provided for added convenience

**Storage:** Room Temperature

R: 20/21/22-36/37/38-42 S: 22-26-36





Genomic DNA purified by GenElute™ Blood Genomic DNA kits is suitable for restriction enzyme digestions.

Figure 1. Restriction Enzymes, EcoR I and Hind III were used to digest genomic DNA isolated with GenElute™ Blood Genomic DNA kit. Whole blood was collected in 3 different anticoagulants: EDTA, Heparin, and Sodium Citrate. A 100 ng aliquot of genomic DNA from each anticoagulant was initially digested with EcoR I (5 units per 1  $\mu$ l digested at

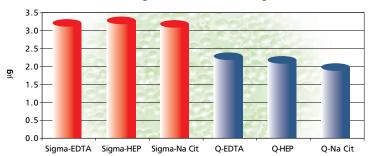
U = Undigested

E = EcoRIH = Hind III 37 °C for 1.5 hours) and Hind III (10 units per 1 µl digested at 37 °C for 1.5 hours) followed by electrophoresis (50 ng/lane) on a 0.8% agarose gel. Ladder (M) used was Lambda Hind III (Product Code D 9780).

E = EDTAH = Heparin NC = Sodium Citrate

## A. Sigma Supplier Q H NC E H NC M

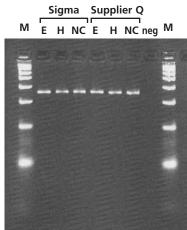
В. **DNA Isolation using 3 Different Anti-Coagulants** 



Whole Blood collected in three different anticoagulants was isolated with the GenElute™ Blood Genomic DNA kit and Supplier Q to obtain genomic DNA.

Figure 2A. Purified genomic DNA from whole blood collected in vacutainer tubes, each containing a different anticoagulant (EDTA, Heparin and Sodium Citrate). Samples were isolated with either the GenElute™ Blood Genomic DNA kit or Supplier Q following both protocols in detail. The genomic DNA (100 ng/lane) was analyzed on a 0.8% agarose gel to show overall comparability with the main kit supplier. The whole blood (200 µl per sample) used was obtained from a human donor. Marker (M) used was Lambda Hind III (Product Code D 9780).

Figure 2B. Yields of genomic DNA compared with leading Supplier Q from 3 different anticoagulants. The amount of DNA was determined measuring absorbance at  $(A_{260}/A_{280})$ .



**←** 500 bp

PCR amplification of a 388 bp product isolated from Genomic DNA.

Figure 3. Whole blood collected in 3 different anticoagulants was purified using the GenElute™ Blood Genomic DNA Kit. A 5 µl aliquot of each eluate

E = EDTAH = Heparin NC = Sodium Citrate

**←** 300 bp

was used as template in a 20 µl PCR reaction for 35 cycles. A 5 μl aliquot of each PCR reaction was resolved on a 2% agarose gel. The PCR marker (M) (Product Code <u>P 9577</u>) used ranged from 50 bp to 2 kb.

Product	Product Description	Preps	Quantity
NA2000	GenElute™ Blood Genomic DNA Kit	10	1 kit
NA2010	GenElute™ Blood Genomic DNA Kit	70	1 kit
NA2020	GenElute™ Blood Genomic DNA Kit	350	1 kit



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#### **GENOMIC DNA PURIFICATION**

#### **GenElute™ Bacterial Genomic DNA Kit**

#### For purification of genomic DNA from a variety of cultured bacteria.

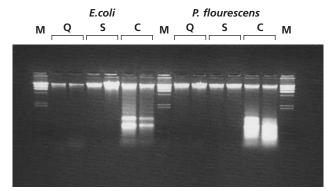
Sigma's GenElute™ Bacterial Genomic Kit provides a simple and convenient technique to isolate high quality DNA from both Gram -(Fig. 1) and Gram + bacteria (Fig. 2). This kit combines the advantages of a silica-based system with a microspin format, eliminating the need for expensive resins and hazardous organic compounds. Bacteria are first incubated with the appropriate enzymes to ensure efficient cell lysis and DNA release from the cells. The bacteria are then lysed in a chaotropic salt-containing solution. DNA is bound to the silica-based membrane and the remaining lysate is removed by centrifugation. After washing to remove contaminants, the DNA is eluted with buffer into a collection tube. Eluted DNA can be up to 50 kb in length (Fig. 3) and is suitable for downstream applications such as restriction endonuclease digestions, PCR, and Southern blots.

The GenElute™ Bacterial Genomic DNA Kit contains all of the reagents needed to purify genomic DNA from Gram - bacteria (Fig. 3). However lysozyme (Product Code <u>L 7651</u>), which must be purchased separately, is needed for most Gram + bacteria to thoroughly lyse the thick peptidoglycan cell walls. A Gram + Lysis Solution is provided with the GenElute™ kit as a diluent for preparing the lysozyme stock solution.

#### **Features and Benefits**

- Protocols provided for Gram + and Gram bacteria
- High quality genomic DNA in less than 2 hours
- Purified DNA has an A<sub>260</sub>/A<sub>280</sub> ratio between 1.6 and 1.9
- $\bullet$  Typical DNA yields of 15  $\mu g$  20  $\mu g$  (See Table 1)
- Lysozyme diluent & RNase A Solution provided for added

**Storage:** Room Temperature R: 20/21/22-36/37/38-42 S: 22-26-36



Comparison of Gram - bacteria genomic DNA isolation kits.

Q = Supplier Q S = Sigma C = Supplier C

Figure 1. Agarose gel analysis of genomic DNA isolated from the indicated Gram - bacteria prepared using the GenElute™ Bacterial Genomic

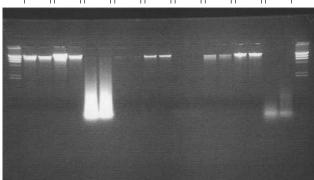
DNA Kit versus kits from other suppliers. Equal proportions of DNA were resolved on a 1%, 1X TBE agarose gel. The Lambda Hind III ladder (Product Code D 9780) was used as a size standard (M).

Table 1: Typical DNA Yields with the GenElute™ Bacterial Genomic DNA Kit.

Source	Type of Media	Amount of Overnight Culture	OD <sub>600</sub> per ml Overnight Culture*	Typical DNA Yield (with RNase Treatment)**
Escherichia coli, ATCC# 11775	Terrific broth (Product Code T 9179)	0.8 ml	12.5	20 μg
Escherichia coli, ATCC# 11775	LB broth (Product Code L 7658)	1.5 ml	5	20 μg
Escherichia coli DH10B	LB broth (Product Code L 7658)	1.0 ml	5	15 μg
Pseudomonas fluorescens, ATCC# 13525	Terrific broth (Product Code T 9179)	0.8 ml	16	25 μg
Pseudomonas fluorescens, ATCC# 13525	Nutrient broth (Product Code N 7519)	1.5 ml	2	20 μg
Bacillus subtilis, ATCC# 6051	Todd Hewitt broth (Product Code T 1438)	1.5 ml	6	25 μg
Streptococcus mutans, ATCC# 35668	Todd Hewitt broth (Product Code T 1438)	1.5 ml	1.3	15 μg***
Staphylococcus epidermidis, ATCC# 14990	Nutrient broth (Product Code N 7519)	1.5 ml	2	8 µg****

- \* Values adjusted for dilution factor.
- \*\* Based on performing two 200  $\mu l$  elutions.
- \*\*\* Lysozyme Solution was supplemented with 250 units/ml of Mutanolysin (Product Code M 9901).
- \*\*\*\* Lysozyme Solution was supplemented with 200 units/ml of Lysostaphin (Product Code L 7386).





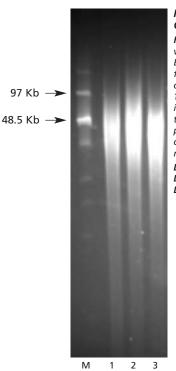
#### Comparison of Gram + bacteria genomic DNA isolation kits.

Figure 2. Agarose gel analysis of genomic DNA isolated from the indicated Gram + bacteria prepared using the GenElute™ Bacterial Genomic DNA Kit versus kits from other suppliers. Equal proportions of DNA were resolved on a 1%, 1X TBE agarose gel. The Lambda Hind III ladder (Product Code <u>D 9780</u>) was used as a size standard (M).

Q = Supplier Q

S = Sigma

C = Supplier C



#### PFGE of Bacterial gDNA isolated with GenElute Bacterial gDNA Kit

Figure 3. Purified genomic DNA was isolated from various bacterial species using the GenElute™ Bacterial Genomic DNA kit. A 1 µg aliquot of DNA from each respective bacterial sample was resolved on a 1% agarose gel in 0.5X TBE at 150 volts for 16 hours using a BioRad CHEF DRII system. The initial pulse time was 2 seconds, the final pulse time was 13 seconds, the start ratio was 1.0, pump speed was set at 70, and PFGE was carried out at 4 °C. M represents the 0.1-200 kb Pulse marker (Product Code <u>D 2291</u>).

Lane 1: E. coli Lane 2: P. fluorescens Lane 3: B. subtilis

#### **ORDERING INFORMATION**

Product	Product Description	Preps	Quantity
NA2100	GenElute™ Bacterial Genomic DNA Kit	10	1 kit
NA2110	GenElute™ Bacterial Genomic DNA Kit	70	1 kit
NA2120	GenElute™ Bacterial Genomic DNA Kit	350	1 kit

Order: 1.800.325.3010

5.583

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1.800.

Service:

Technical