Genomic DNA Purification

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. 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, specially formulated for amplification directly from the extract. This formulation uses an antibody based hot start DNA polymerase for specific amplification. The kit also includes a PCR reaction mix, specially formulated for use with plant extract.

Genomic DNA is extracted from 0.5-0.7 cm plant leaf disks that have been cut with a standard paper punch and 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 or Extract-N-Amp PCR Mix.

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 master mix for use with plant extract
- Antibody inactivated DNA polymerase for highly specific PCR amplification of genomic DNA
- REDExtract-N-Amp requires no loading buffers or tracking dyes for gel analysis
- Compatible with high-throughput requirements for genetic analysis of plants
- Plant extract storage at 4 °C for up to 6 months

Storage: −20 °C
Shipped in wet ice
R: 36/37/38 S: 26-36
Sequence determination for GAPDH tomato leaf PCR product. Direct sequence from tomato leaf PCR products generated using Extract-N-Amp Plant Kit. The product was sequenced directly using BigDye terminator v.3.1 chemistry. Sequencing reactions were analyzed on an ABI 3730xl.

**PCR Analyses of Genomic DNA Extracted from Various Plant Sources**

Genomic DNA was extracted from 0.5 cm leaf disks. DNA was extracted then amplified using the specially formulated hot start PCR ReadyMix. 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.

**Ordering Information**

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**Stability of Plant Extracts at 37 °C**

Stability of plant extracts at 37 °C. 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 (Catalog Number 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.
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 genomic DNA from whole blood and amplify targets of interest by PCR. 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 master mix, especially formulated for amplification directly from the extract. This formulation uses an antibody based hot start DNA polymerase 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 Mix 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
- Antibody inactivated DNA polymerase included for highly specific PCR amplification of genomic DNA
- Compatible with any format (single tube, 96-well, etc.)
- No phenol/chloroform extraction required
- Blood extract storage at 4 °C for up to 6 months

**Storage:** –20 °C

Shipped in wet ice

R: 34  S: 26-27-36/37/39

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There is no heating in this Extract-N-Amp, making it automation friendly.
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.

### PCR Analysis of Genomic DNA Isolated from Blood

The PCR analysis was performed using the REDExtract-N-Amp Blood PCR Kit. 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

Stability of blood extracts at 37 °C. Stability of Extract-N-Amp Blood Extracts: Blood was drawn from 2 human volunteers into Vacutainer® tubes containing EDTA. Extractions were performed in duplicate providing 4 samples total. Samples were stored at 37 °C and 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. DNA for the standards was 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 for 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.

### Ordering Information

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Genomic DNA Purification

**Extract-N-Amp™ Tissue PCR Kits**

*From tissue or cells to PCR in 15 minutes*

The Extract-N-Amp Tissue PCR Kits provide all the reagents necessary to rapidly extract DNA from a wide variety of cells and tissues and amplify targets of interest by PCR. A novel extraction method eliminates the need for long enzymatic digestions or homogenization. The kit also includes a specially formulated hot start PCR ReadyMix™ for amplification directly from the extract. The PCR ReadyMix comes in two formulations: Extract-N-Amp PCR ReadyMix and REDExtract-N-Amp™ PCR ReadyMix. The REDExtract-N-Amp PCR ReadyMix contains an inert dye that acts as a tracking dye and allows for convenient loading of PCR reactions onto agarose gels for analysis.

The kit comes with validated protocols to extract and amplify genomic DNA from mouse-tails, hair, animal tissue, saliva and buccal swabs. In a typical procedure, genomic DNA is extracted from a tissue sample that has been incubated in the tissue preparation solution and extraction solution for 10 minutes at room temperature. The sample is heated to 95 °C for 3 minutes and then mixed with a third solution 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 or Extract-N-Amp PCR ReadyMix included in the kit.

**Feature and Benefits**

- **Fast** – rapid extraction of genomic DNA for PCR in 15 minutes
- **Convenient** – no long enzymatic digestions
- **Practical** – perfect for quick genomic DNA isolation for genotyping
- **Flexible** – protocols available for mouse-tails, hair, animal tissue, saliva and buccal swabs
- **Specific** – hot start antibody for highly specific PCR amplification of genomic DNA

**Storage:** –20 °C

Shipped in wet ice

R: 36/37/38-42/43  S: 26-36

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### Overview of Extract-N-Amp™ Tissue PCR Kit Procedure

1. **Tissue Sample** + **Tissue Preparation Solution**
2. Incubate with **Extraction Solution** for 10 minutes at room temperature.
3. **Heat** at 95 °C for 3 min.
4. **Add Neutralization Solution.**
5. Mix aliquot with REDExtract-N-Amp™ or Extract-N-Amp™ PCR ReadyMix™ and primers.
6. **Transfer to thermal cycler.**
7. **PCR**
8. **Directly load amplified PCR product on gel.**

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**Sigma.com/pcr**

ORDER: 800-325-3010  TECHNICAL SERVICE: 800-325-5832
Genomic DNA Purification

Sequence determination for 1181 bp Interleukin 1 Beta mouse-tail PCR product. The PCR product was purified with the GenElute™ PCR Clean-Up Kit (Catalog Number NA1020). The DNA extraction and PCR were performed using Sigma’s Extract-N-Amp™ Tissue PCR Kit. The sequence was obtained using the ABI BigDye® Terminator Chemistry and the same primers as for the original PCR. Reaction products were resolved on an ABI 310.

PCR Analysis of Genomic DNA Extracted from Various Samples Using Sigma’s Extract-N-Amp Tissue PCR Kit

Stability of Mouse Tail Extracts at 37 °C

Stability of mouse tail extracts at 37 °C. Mouse-tail samples were extracted according to the procedure in the Technical Bulletin for the Extract-N-Amp Tissue PCR Kit. The remaining mouse-tail tissue was removed from the samples for storage. 4 µl aliquots were analyzed immediately by quantitative PCR with SYBR® Green detection on an ABI Prior® 7700. DNA standards for quantitative PCR were purified DNA prepared from mouse tails using the GenElute Mammalian Genomic DNA Kit (Catalog Number GI1070) and stored as single use aliquots at –20 °C. The mouse-tail extracts were stored at 37 °C (accelerated storage). Quantitative PCR was repeated after 3 weeks, 5 weeks and 2 months from extracts at 37 °C. Results for storage at 37 °C are shown. These results suggest that extracts will be stable for at least 6 months at the recommended storage temperature of 4 °C.

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Inquire for bulk and high-throughput needs.
Genomic DNA Purification

**SYBR® Green Extract-N-Amp™ Tissue PCR Kit**

The SYBR Green Extract-N-Amp Tissue PCR Kit contains all the reagents needed for rapid extraction, amplification and detection of genomic DNA from mouse-tails and other animal tissues, buccal swabs, hair shafts and saliva.

The SYBR Green Extract-N-Amp Tissue PCR Kit offers an innovative extraction system that eliminates the need for either long enzymatic digestions or homogenization. The product includes a specially formulated Hot Start SYBR Green PCR ReadyMix™ for amplification and quantitation directly from the extract.

**Procedure:**
DNA is rapidly extracted from a tissue by incubating the sample with a mixture of the Extraction Solution and the Tissue Preparation Solution at room temperature for 10 minutes. After a 3-minute heat denaturing step, an equal volume of Neutralization Solution B is added to the extract to neutralize inhibitory substances. The extract is ready for real-time PCR in any plate-based real-time thermal cycling system in less than 15 minutes!

**Application:** Ideal for genotyping, gene copy number experiments, and amplifying and quantifying DNA from multiple tissue sample types.

**Features and Benefits**
- Novel – all liquid, single-step extraction of genomic DNA for quantitative PCR (qPCR)
- Fast – tissue to qPCR in 15 minutes
- Convenient – no long enzymatic digestions and no column purifications
- Simple – rapid, easy-to-follow protocol
- Sensitive – specially formulated Hot Start SYBR Green PCR ReadyMix for highly specific PCR amplification and quantitation of genomic DNA
- Safe – no organic extraction with hazardous chemicals

**Storage:**
-20 °C  
R: 42  S: 36/37-45
Distinguish Differences in Gene Copy Number

Distinguish differences in gene copy number. SYBR Green Extract-N-Amp used to distinguish between 1-2 gene copies. Extracts were prepared following the standard protocol for the SYBR Green Extract-N-Amp Tissue PCR Kit from 8 mouse tails that contained either a single or double copy of the Diap2 gene. Two single-gene PCR reactions were run on each extract using the 2× Extract-N-Amp SYBR Green PCR ReadyMix.

Flexible Enough for A Wide Variety of Tissue Types

Flexible enough for a wide variety of tissue types. Quantitative PCR of DNA isolated from a variety of tissue types and sources. The Extract-N-Amp Tissue PCR Kit was used to extract and amplify genomic DNA from various sources. The extracted DNA was then amplified using the specially formulated Hot Start SYBR Green PCR ReadyMix.

High Sensitive SYBR Green PCR ReadyMix

High sensitive SYBR Green PCR ReadyMix. Quantitative PCR of DNA isolated from a series of decreasing extract dilutions. DNA was extracted from a mouse-tail snip following the standard protocol for the SYBR Green Extract-N-Amp Tissue PCR Kit. The extract was diluted in decreasing three-fold increments, from 100% extract to 0.14% extract. As depicted above, message can be clearly detected even with the most dilute extract.

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Genomic DNA Purification

Extract-N-Amp™ Seed PCR Kits

Rapid genomic DNA extraction from seeds

The Extract-N-Amp Seed PCR Kits can be used to extract seed genomic DNA that is suitable for PCR in just 15 minutes, using a straightforward extraction protocol. The PCR product that is amplified using the Extract-N-Amp Seed PCR Kit is suitable for direct sequencing. No phenol/chloroform extraction or alcohol precipitation is required. The DNA extracted by the Extract-N-Amp Seed PCR Kit is stable for at least 6 months at 4 °C, allowing for multiple re-assays.

Features and Benefits
- Fast – 15 minute extraction of genomic DNA for PCR
- Flexible – compatible with a wide variety of seed sources
- Specific – Hot Start antibody for highly specific PCR amplification of genomic DNA
- Simple – no column purification or centrifugation required

Storage: –20 °C
R: 36/37/38-42  S: 26-36

Identify Specific Genes Before Planting Seeds

Genomic DNA was extracted from seeds using the protocol as described in the Extract-N-Amp Seed Technical Bulletin. All extracts were then amplified using the specially formulated JumpStart™ PCR ReadyMix™ and PCR primers multiplexed for both a universal chloroplast gene (~400-700 bp) and the acetylcoenzyme A carboxylase gene specific to wheat (964 bp).

For Plant Seeds

Identify specific genes before planting seeds. Genomic DNA was extracted from seeds using the protocol as described in the Extract-N-Amp Seed Technical Bulletin. All extracts were then amplified using the specially formulated JumpStart™ PCR ReadyMix™ and PCR primers multiplexed for both a universal chloroplast gene (~400-700 bp) and the acetylcoenzyme A carboxylase gene specific to wheat (964 bp).

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