



**Genomic DNA Extraction Kit
INSTRUCTION MANUAL**

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Introduction

Fast ID provides high yields of exceptionally pure DNA from a wide variety of agricultural and food products. Fast ID is especially designed to provide high yields of DNA from moderately and highly processed foods. The extracted and purified DNA is suitable for any molecular biology procedure, including but not limited to PCR (Polymerase Chain Reaction) amplification, restriction digestion, cloning, and sequencing. Genetic ID NA recommends the use of Fast ID with all of its PCR-based GMO detection kits for optimal amplification and minimal inhibition.

Fast ID is able to extract DNA from a wide variety of sample types. The Fast ID Genomic DNA Extraction Kit is designed for whole grains, fruits, and vegetables, and the Fast ID Ultra DNA Extraction Kit (Catalog No. K6-0001-050 or -200) is designed for processed and/or multi-ingredient foods. Fast ID contains powerful denaturants and DNA-selective reagents that are specifically designed to prevent DNA degradation and to remove contaminants that might inhibit the PCR reaction or generate artifactual DNA template-primer interactions.

Kit Components

Kit Name	Catalog Number	
Fast ID Genomic DNA Extraction	50 extractions	200 extractions
<ul style="list-style-type: none">• Proteinase K• Genomic Lyse buffer• Genomic Bind buffer• Genomic Wash buffer• DNA Binding Columns• 1xTE (10 mM Tris-HCl, 1 mM Na-EDTA pH 8.0)	K1-0001-0050	K1-0001-0200

Storage Conditions

All components of the Fast ID kit should be stored at room temperature (20-25 °C) and are stable for one year under these conditions.

Recommended Equipment and Reagents (not supplied)

- 60-65 °C incubator
- Vacuum manifold (suggested model: QIAvac 24, capable of holding 24 columns)
- Microcentrifuge (capable of carrying 1.5 ml and 2 ml microcentrifuge tubes)
- 75% ethanol
- chloroform (optional reagent)
- Molecular biology reagent grade water

Introduction to the Protocol

Unlike many DNA extraction kits, Fast ID is applicable to a wide range of sample types. Through experimentation, DNA from various sample types can be successfully extracted with Fast ID. Tolerance for weights and volumes are +/- 10%.

General Overview of the Protocol

1. Prior to using the protocol, Genetic ID recommends that care be taken in homogenizing the sample in a manner that minimizes the risk of inadvertently contaminating the sample. Contamination can occur using improperly cleaned equipment or using poor laboratory practices during homogenization, weighing and labeling of the subsample.
2. The weighed subsample is incubated with the Genomic Lyse buffer, which lyses the cells of the subsample and solubilizes proteins, DNA, and other cellular constituents. Proteinase K is added to digest protein in the sample.
3. Genomic Bind buffer is added to the DNA-containing supernatant and then passed through a column that binds the DNA.

Genomic DNA Extraction

Genomic Lyse Procedure

Dissolve the complete amount of Proteinase K supplied in this kit with highly purified water, such as Molecular Biology Reagent grade water (Sigma) or Cell Culture Grade (Gibco), to obtain 10 mg/ml Proteinase K solution in the amounts designated below:

Kit Size	Amount of Purified Water
50 extractions	500 μ l
200 extractions	2000 μ l

- 1) For each extraction, take 1,000 μ l of Genomic Lyse buffer and premix with 10 μ l of Proteinase K solution.

Note: If precipitates occur in the Genomic Lyse buffer (usually due to cold temperature) then the buffer must be warmed to 20 to 30 °C and thoroughly mixed in order to completely solubilize its contents.

- 2) In a labeled 2 ml vial add 200 mg of ground and homogenized sample and mix with 1,000 μ l Genomic Lyse buffer premixed with Proteinase K (#1).
- 3) Vortex thoroughly until a homogeneous slurry is obtained.
- 4) Incubate at 65 °C for 10 to 30 minutes.
- 5) Spin at about 10,000 rpm for 5 minutes in a microcentrifuge.
- 6) Take 500 μ l of supernatant and transfer into a new labeled 2 ml vial.

Note: If less volume is available, transfer as much as possible without taking sediment. If material floats on the surface, avoid carrying it over into the vial.

- 7) Add an equal amount of Genomic Bind buffer and vortex briefly. If, in very rare cases, the indicator color changes, refer to the section on troubleshooting.
- 8) Spin at 10,000 rpm for 5 minutes in a microcentrifuge.
- 9) Pass the supernatant through the DNA Binding Column. This can be accomplished either with a vacuum manifold having luer lock fittings or by centrifugal force.

Note: Clean any spilled buffer. Salts contained in the buffer solution can cause corrosion of laboratory surfaces.

- 10) Wash with 1,000 μ l of Genomic Wash buffer using the vacuum manifold or 800 μ l if using centrifugation. Then collect the flow through in the vials packed with the columns.
- 11) Wash three times with 1,000 μ l 75% ethanol if using vacuum manifold or 800 μ l if using centrifugation.
- 12) Spin for a few seconds at high speed (~ 10,000 rpm) in a microcentrifuge.
- 13) Place the column into a 1.5 ml vial.
- 14) Add an appropriate amount of 1xTE depending on the intended downstream application. For most applications 100 μ l is adequate.
- 15) Incubate for 5 to 10 minutes at 65 °C.
- 16) Spin at 10,000 rpm for 5 to 30 seconds in a microcentrifuge and collect the eluted DNA in the 1.5 ml vial. Discard the column.

Note:

1. If DNA yield is low, please refer to the troubleshooting section.
2. For complex samples, an additional chloroform separation at Step #5 can be helpful. Add an equal amount of chloroform and spin as described in Step #5. Remove the supernatant for further use as described in Step #6.

Genomic DNA Extraction

Lysis

Sample Preparation (200 mg)

Add 1ml Genomic Lyse buffer

Incubate @ 65°C for 10 to 30 minutes

Centrifuge @ 10,000 rpm for 5 min

Binding

Mix 0.5 ml of the supernatant with 0.5 ml Genomic Bind buffer

Centrifuge @ 10,000 rpm for 5 min

Pass supernatant through DNA Binding Column

Washing

Wash column with 0.8 – 1.0 ml Genomic Wash buffer

Wash column 3 x with 0.8 – 1.0 ml 75% Ethanol

Dry spin the column

Elution

Elute and collect DNA from column with 0.1-0.2 ml of 1x TE

Health and Safety

Material Safety Data Sheets (MSDS) for all components of Fast ID can be requested by emailing Genetic ID at techsupport@fastiddna.com or by downloading from our website: www.fastiddna.com.

Product Warranty

This product is guaranteed by Genetic ID to perform as it is described in this Instruction Manual. Genetic ID reserves the right to alter any product or element within this product to improve operating ease and performance. It is the responsibility of the purchaser to ascertain the suitability of the kit to meet their needs. If you are unsatisfied for any reason with this product, please contact Technical Assistance at: techsupport@fastiddna.com. If the product has failed due to causes other than misuse, we will exchange the product.

Product Use Limitations

Genetic ID DNA extraction kits are intended for research purposes only. These kits are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly approved for that purpose by the U. S. Food and Drug Administration (FDA) in the USA or the appropriate regulatory body in the country of use. All kit components should be handled with care and stored under the conditions described in this manual. Waste should be disposed of properly in accordance with all local and regional laws.

Quality Control

Fast ID was developed by Genetic ID NA, Inc. Fast ID is used in our testing laboratory which is accredited to the ISO 17025 standard. All components of Fast ID were validated with appropriate sample types prior to shipping.

Technical Assistance

For technical assistance contact Genetic ID Customer Service at techsupport@fastiddna.com or call +1 641 472 9979.

Ordering Information

Kits may be ordered online on our website: www.fastiddna.com. Or, contact Genetic ID at sales@fastiddna.com or call +1 641 472 9979. Please see Kit Components on Page 3 for catalog number.

Disclaimer

The information above is based on our current knowledge and is believed to be accurate. However, we make no guarantee for any specific product features or any other guarantee, expressed or implied. We assume no liability resulting from its use and we shall not establish a legally valid contractual relationship. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no way shall Genetic ID NA, Inc. and/or its affiliates be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Genetic ID NA, Inc. has been advised of the possibility of such damages.

If you open this package (the kit), this action indicates that you agree not to attempt to reverse engineer or otherwise learn the technology behind the DNA extraction technology contained herein.

TroubleShooting Fast ID Genomic DNA Extraction Kit

<p><i>DNA yield unsatisfactory (via UV or fluorometric determination)</i></p>	<ul style="list-style-type: none"> • Make sure the sample is thoroughly ground and homogenized, i.e., for grain, the sample should have a flour-like consistency. For leaf tissues, use of bead beaters or wet grinding with Genomic Lyse buffer is recommended. • Thorough vortexing at Step #3 is critical for optimal yield and DNA quality. • Optimal incubation times are at the high end of the indicated range. • Make sure that all supernatant is passed through the DNA binding column in Step #9. • Reducing the column flow-through speed during DNA binding (Step #9) increases DNA yield. If the centrifugation method is used, about 1,000 rpm for up to 5 minutes is recommended. Or alternatively, for this step only, flow-through by gravity may be useful. In this case, the sample should pass through the DNA Binding Column within 5 to 15 minutes. Note: washing steps can always be performed at high flow-through speed in order to save time. • Sample size can be increased up to 1 g as long as a corresponding increase in Genomic Lyse buffer volume is used. • Increasing Proteinase K may increase yield for some samples. • For some samples an additional chloroform purification at Step #5 increases yield. Add an equal amount of chloroform and spin as described in Step #5. Remove the supernatant for further use as described in Step #6.
<p><i>Slow flow speed through the DNA Binding Column</i></p>	<ul style="list-style-type: none"> • The solution in Step #9 must be transparent in order to pass through the column. Increasing the centrifugation time at Step #8 and/or an additional chloroform purification at Step #5 is helpful. Add an equal amount of chloroform and spin as described in Step #5. Take the supernatant for further use as described in Step #6.
<p><i>DNA extraction procedure takes too long</i></p>	<ul style="list-style-type: none"> • In general, DNA extraction of 5 samples can be achieved within 2 hours using a vacuum manifold (preferred solution) or centrifugation. The exception is described for low yield DNA (in this troubleshooting section).
<p><i>DNA Binding column membrane is colored</i></p>	<ul style="list-style-type: none"> • Wash two times with Genomic Wash buffer followed by three washes with 75% ethanol and one wash with 100% ethanol in order to remove colored compounds from the DNA bound to the membrane.
<p><i>PCR Inhibition</i></p>	<ul style="list-style-type: none"> • PCR inhibition can occur due to salt carry-over in the DNA. It is very important to ensure proper washing with 75% ethanol during the ethanol washing steps. Always fill the DNA Binding Column to the top with 75% ethanol. At the second washing step it is recommended to wash the upper rim of the DNA Binding Column. • PCR inhibition can occur as a result of sample type rich in inhibitory compounds. In this case, an additional washing step with Genomic Wash buffer is recommended. Alternatively, an additional chloroform purification at Step #5 can be helpful. Add an equal amount of chloroform and spin as described in Step #5. Remove the supernatant for further use as described in Step #6.
<p><i>DNA fragmentation</i></p>	<ul style="list-style-type: none"> • Minimize vortexing. The critical vortex step is at Step #3. At all other steps, gentle inversion of the tube about 10 times is an alternative to vortexing.
<p><i>If RNA contamination is suspected</i></p>	<ul style="list-style-type: none"> • Premix the Genomic Lyse buffer with 10 µl of RNase (1mg/ml), vortex briefly, add 1,000 µl of premixed buffer to the sample, vortex briefly, and incubate for 10 to 30 minutes at room temperature. Then add 10 µl of Proteinase K solution and continue with Step #3.

