Genomic DNA Magnetic Bead Kit – KOA0912

Rockland's Genomic DNA Magnetic Bead Kit is designed to purify genomic DNA from mammalian tissues and bacteria. Paramagnetic beads with uniform particle size efficiently bind DNA, resulting in high yields of DNA with minimal RNA, proteins, nucleases, and other cellular contaminants. The kit is intended for manual purifications using a magnetic separator. The protocol can be easily customized to optimize sample yield and quality depending on the type of tissue or bacteria (See Section XI for customization options). The kit contains magnetic beads and reagents sufficient for 100 samples. The uniform size and surface area of beads ensure highly reproducible results and improved performance compared to magnetic beads from alternative suppliers.

I. KIT COMPONENTS

Contents	Catalog Number	Size
Magnetic Beads Solution	KOA0912E	2 x 1.1 mL
Lysis Solution	KOA0912A	22 mL
Proteinase K Solution	KOA0912B	2.1 mL
RNase A Solution	KOA0912C	2.1 mL
Binding Solution concentrate	KOA0912D	20 mL
Wash Solution 1 concentrate	KOA0912F	14 mL
Wash Solution 2 concentrate	KOA0912G	18 mL
Elution Solution	KOA0912H	22 mL

II. GENOMIC DNA MAGNETIC BEAD CHARACTERISTICS

Bead Mean Diameter	5 µm
Bead Concentration	12.5 mg/mL
Binding Capacity	Varies with sample type

III. STORAGE AND STABILITY CONDITIONS

Upon delivery of the kit, separate the kit components and store as directed. Remove the RNase A Solution and Proteinase K Solution vials and store at -20°C. Remove the Magnetic Beads Solution (KOA0912E) and store at 4°C. Do not freeze the magnetic beads solution, because they may become damaged. Keep the beads in liquid suspension during storage as drying will result in reduced performance. All other kit reagents may be stored at room temperature (20-25°C). Do not use after the printed expiration date.

IV. SAFETY INSTRUCTIONS

Use appropriate protective equipment (including but not limited to gloves, lab coats, and safety glasses) when collecting tissues and bacteria. The Binding Solution concentrate (KOA0912D) and Wash Solution 1

concentrate (KOA0912F) contain guanidine hydrochloride, which can be irritating to eyes and skin. Always wear gloves, lab coats, safety glasses, and/or other protective equipment when using these solutions. **Refer to Safety Data Sheet for further information**.

V. INTRODUCTION

The Genomic DNA Magnetic Beads Kit process uses a simple, efficient, magnetic bead-based procedure for genomic DNA purification, as illustrated below in **Figure 1**:

- **Sample Digestion:**
  Incubate crude sample with Lysis Solution (KOA0912A), Proteinase K Solution (KOA0912B), and RNase A Solution (KOA0912C) to digest cells and denature proteins and RNA. Add the resulting solution to genomic DNA magnetic beads.
- **Binding:**
  Add binding solution concentrate (KOA0912D) to bind DNA to the beads. Capture DNA-bead complex using magnetic separator.
- **Washing:**
  Remove cell debris and wash beads twice with wash solution.

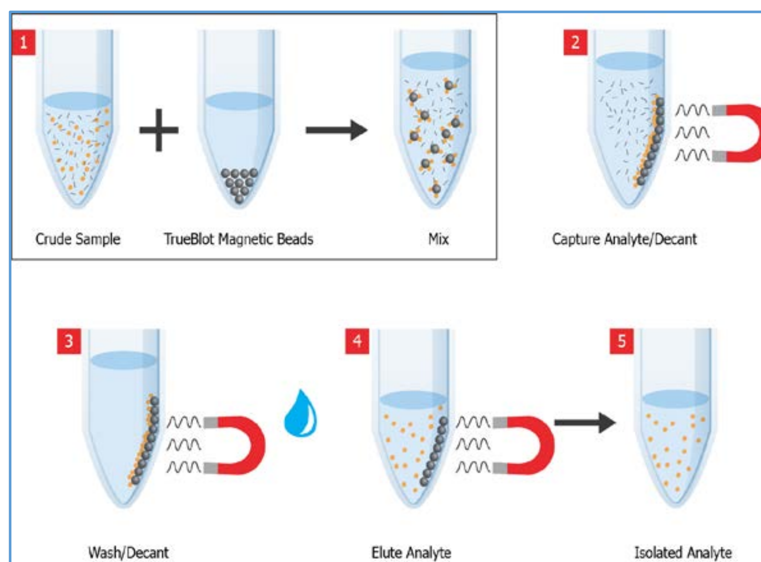

Figure 1

- **Elution:**
  Add elution buffer and elute purified DNA. Transfer DNA to a new tube.
- **Downstream Applications:**
  Pure, high-quality isolated genomic DNA may then be used for downstream procedures such as PCR and qPCR, or stored long-term.

VI. REQUIRED EQUIPMENT AND REAGENTS

- Disposable gloves and other protective equipment
- Micro-pipettes with disposable plastic tips
- 1.5 mL sterile plastic microcentrifuge tubes
- 4°C refrigerator
- -20°C freezer
- 96-100% Ethanol
- Tissue disruption equipment
- Vortexer
- Heating block, thermomixer, or water bath capable of 65°C

- Magnetic separators (Cat # TMS-15-50; TMS-06; TMS-32)
- Minicentrifuge
- Lysozyme buffer (Gram-positive bacteria only):
  25mM Tris-HCl pH 8.0, 2.5 mM EDTA, 1% Triton X-100, add fresh Lysozyme to 20 mg/mL concentration immediately before use.

VII. LIMITATIONS AND PRECAUTIONS

Initial handling of sample tissue can significantly affect the yield and quality of resulting DNA. To avoid degrading the DNA, use fresh sample material, or immediately freeze samples at -20°C to -80°C until purification. Avoid freezing and thawing samples repeatedly. Overall DNA yield, quality and test reproducibility may vary depending on sample type and amount, age, and condition before and after storage.

VIII. REAGENT PREPARATION

Before the first use of the kit, add 96-100% Ethanol to the **Binding Solution concentrate (KOA0912D)**, **Wash Solution 1 concentrate (KOA0912F)**, and **Wash Solution 2 concentrate (KOA0912G)** as specified below. Mark the bottle to indicate that ethanol has been added. Wear gloves when handling the reagents (see Safety Instructions in Section IV).

- Binding Solution: Add 12 mL 96-100% Ethanol
- Wash Solution 1: Add 42 mL 96-100% Ethanol
- Wash Solution 2: Add 42 mL 96-100% Ethanol

Note: Before each use, check for any precipitate formation in the solutions. If observed, shake to re-dissolve any precipitates.

IX. KIT PROTOCOL

Sample Preparation

Mammalian Tissues:

- Cut fresh or frozen tissues into small pieces using dissection scissors, razor, mortar and pestle with liquid nitrogen, homogenizer, or similar. Cut tissue samples quickly or on ice to avoid extended times at room temperature.
- Weigh out up to 15 mg of tissue pieces.
- Collect tissue pieces into a 1.5 mL microcentrifuge tube (not provided) pre-filled with 200 µL **Lysis Solution (KOA0912A)**.

Gram-negative Bacteria:

- Add up to 2 x 10^9 Gram-negative bacterial cells (about 1 mL of overnight culture) to a 1.5 mL microcentrifuge tube (not provided).
- Centrifuge 10 minutes at 5,000 x g to pellet the cells, and discard the supernatant.
- Add 200 µL **Lysis Solution (KOA0912A)** and vortex or pipette up and down to resuspend the pellet.

Gram-positive Bacteria:

- Add up to 2 x 10^9 Gram-positive bacterial cells (about 1 mL of overnight culture) to a 1.5 mL microcentrifuge tube (not provided).

- Centrifuge 10 minutes at 5,000 x g to pellet the cells, and discard the supernatant.
- Add 400 µL **Lysozyme buffer** with fresh Lysozyme and vortex.
- Incubate 1 hour at 37°C, vortexing occasionally.
- Centrifuge 10 minutes at 5,000 x g to pellet the cells, and discard the supernatant.
- Add 200 µL **Lysis Solution (KOA0912A)** and vortex or pipette up and down to resuspend the pellet.

Procedure

- Add 20 µL of **Proteinase K Solution (KOA0912B)** to the Lysis Solution/sample tube and vortex well. Ensure that the sample is fully submerged in the solution mix.
- Incubate the sample at 55°C for 30 minutes (bacteria) to 1 hour (tissues) until digested, vortexing occasionally or using a thermomixer. For larger sample pieces, lysing for 2 hours or more may be required.
- Pulse spin (~1 second) the sample lysate with a minicentrifuge to remove any condensation from the sides and lid of the tube.
- Add 20 µL of **RNase A Solution (KOA0912C)** and vortex well. Incubate at room temperature for 10 minutes.
- During the RNase A incubation, in a new 1.5 mL tube add 400 µL of 96-100% Ethanol (not provided). Vortex the **Magnetic Beads Solution (KOA0912E)** well to ensure complete resuspension of the beads, and add 20 µL of bead solution to the ethanol. Vortex well.
- After the RNase A incubation, add 300 µL of **Binding Solution** (prepared with ethanol, see section VIII) to the sample lysate and vortex for 3 seconds. Transfer the sample lysate to the ethanol/bead mix and vortex for 5 seconds. Allow the tube to sit at room temperature for ~30 seconds.
- Place the tube on the magnetic separator for 3 minutes. Leaving the tube on the separator, remove and discard the supernatant using a pipette, without disturbing the beads that have collected at the magnet.
- Remove the tube from the magnetic separator and add 500 µL **Wash Solution 1** (prepared with ethanol, see section VIII). Vortex briefly to resuspend the beads and return the tube to the magnetic separator for 2 minutes. Leaving the tube on the separator, remove and discard the supernatant using a pipette, without disturbing the beads that have collected at the magnet.
- Remove the tube from the magnetic separator and add 500 µL **Wash Solution 2** (prepared with ethanol, see section VIII). Vortex briefly to resuspend the beads and return the tube to the magnetic separator for 2 minutes. Leaving the tube on the separator, remove and discard the supernatant using a pipette, without disturbing the beads that have collected at the magnet.
- Pulse spin to remove any wash solution drops remaining on the sides and lid of the tube. Return the tube to the magnetic separator for ~30 seconds. Leaving the tube on the separator, remove and discard any additional supernatant using a pipette, without disturbing the beads that have collected at the magnet.
- Remove the tube from the magnetic separator and add 150 µL **Elution Solution (KOA0912H).** Vortex briefly to resuspend the beads.
- Incubate the sample at 65°C for 10 minutes, vortexing occasionally or using a thermomixer.
- Pulse spin to remove any condensation from the sides and lid of the tube.

- Return the tube to the magnetic separator for 2 minutes. Leaving the tube on the separator, transfer the eluate to a new 1.5 mL tube using a pipette. ***The eluate contains the purified genomic DNA.***

Storing DNA

Store the purified DNA in the Elution Solution at 4°C for immediate use, or at -20°C for long-term storage. To avoid repeated freezing and thawing, store the DNA in aliquots.

X. ANALYSING RESULTS

DNA Yield

- DNA yield can be estimated by UV absorbance. Using a spectrophotometer blanked against the Elution Solution, measure the A260 (DNA absorbance) and A320 (turbidity/cuvette impurity) readings. Use the following equation:

  DNA yield (µg) = (A260 reading – A320 reading) x dilution factor x 50 µg/mL x sample elution volume (mL). Subtracting the absorbance at A320 results in a corrected reading that does not overestimate the DNA quantity.

  For DNA, an A260 of 1.0 = 50 µg/mL when measured in a cuvette with a 10 mm optical path length.

Table 1 below reports the expected yields of genomic DNA obtained from a variety of sample types using the **Genomic DNA Magnetic Beads Kit**:

Sample Type	Starting	DNA Yield
Mouse brain	15 mg	Up to 9 µg
Mouse ear clip	15 mg	Up to 14 µg
Mouse lung	15 mg	Up to 22 µg
Mouse muscle	15 mg	Up to 4 µg
Mouse tail clip	15 mg	Up to 23 µg
E. coli cells	~ 2 x 10^9	Up to 12 µg
Lactobacillus cells	~ 2 x 10^9	Up to 13 µg

Table 1: Expected yield of genomic DNA from various samples types and starting quantities using the **Genomic DNA Magnetic Beads Kit,** standard protocol.

DNA Quality

- Similarly, DNA quality can be estimated by UV absorbance readings. Measure the absorbance at A280 and A230, and correct by subtracting the A320 absorbance. Highly pure DNA has an A260/A280 ratio of ~1.7–2.0, indicating it has minimal contamination by proteins, and an A260/A230 ratio of >1.5, indicating it has minimal contamination by organic compounds and salts. **Figure 2** below displays a spectrophotometric curve of DNA purified from mouse brain tissue, and **Figure 3** shows genomic DNA from various samples analyzed by gel electrophoresis, another indicator of quality:

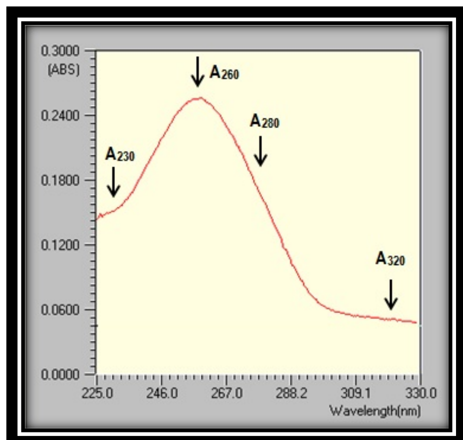

Figure 2. Spectrophotometric curve of high-purity DNA purified from 15 mg of mouse brain tissue using the **Genomic DNA Magnetic Beads Kit** standard protocol.

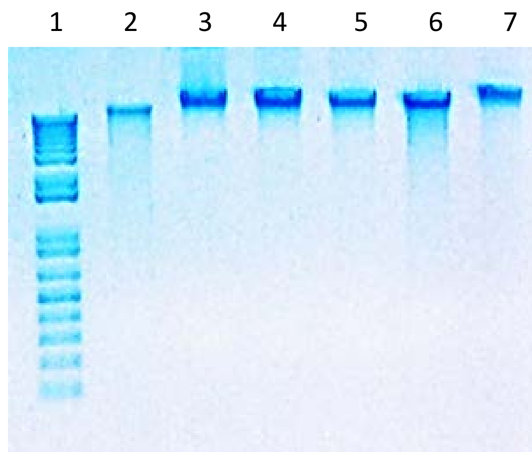

Figure 3. Agarose gel electrophoresis of 450 ng genomic DNA purified using the **Genomic DNA Magnetic Beads Kit** for the following samples: 1 Kb Plus molecular weight ladder (lane 1); Beef (lane 2); mouse brain (lane 3); mouse ear (lane 4); mouse lung (lane 5); mouse tail (lane 6); *E.Coli* cells (lane 7).

XI. PROTOCOL CUSTOMIZATION OPTIONS FOR EXPERIENCED USERS

Many different types of mammalian tissues and bacteria may be used as starting material for DNA purification using the **Genomic DNA Magnetic Beads Kit**. Different sample matrices have very different structures and expected DNA yields (see Section X). As such, the experienced user may wish to adjust various steps in the standard protocol to optimize the results for the desired downstream application. Listed below are suggested customization options.

A. Starting Sample Amount

The amount of starting sample material used directly impacts the amount of DNA purified. Some sample matrices, such as mammalian muscle and heart tissues, generally yield lower DNA amounts due to their fibrous or fatty structure. If a larger quantity of DNA is required for samples like these, a higher amount of initial sample can be used during the sample lysate preparation step, Section IX -Kit Protocol.

Figure 4 below illustrates how increasing the starting sample amount increases the DNA yield:

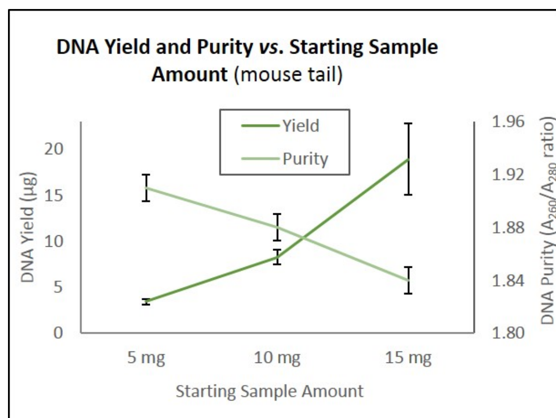

Figure 4: DNA yield (µg) vs. purity (corrected A260/A280 ratio) using starting amounts of 5, 10, or 15 mg of mouse tail tissue and 150 µL elution volume.

Note that increasing the amount of sample greatly increases the yield and slightly decreases the purity ratio of the genomic DNA obtained for this sample matrix (mouse tail). For tissues like these that generally yield higher amounts of pure DNA, decreasing the amount of starting material may be preferable if limited material is available, and/or to obtain higher purity. For this reason, it is useful to optimize the starting sample amount according to the specific tissues being purified by the user, and the intended downstream application. In general, 15 mg of sample material is recommended.

B. Elution Sample Amount

Changing the amount of Elution Solution (KOA0912H) added in section IX under kit protocol procedure, changes the resulting concentration and yield of DNA. Depending on the intended downstream application, a higher concentration of the final sample or a higher overall yield of DNA may be more desirable.

Figure 5 below demonstrates the inverse relationship between concentration and yield:

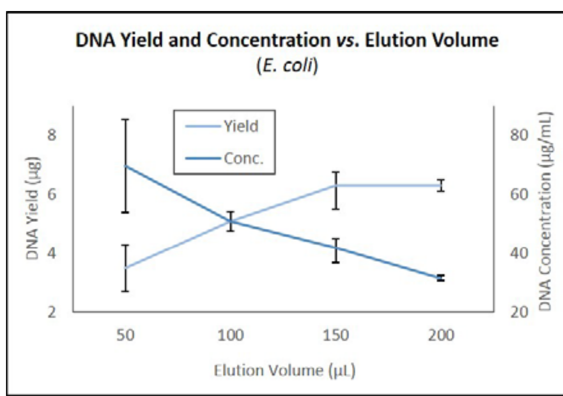

Figure 5: DNA yield (µg) vs. concentration (µg/mL) using ~2 x 10^9 *E.coli* cells with increasing elution volumes.

To increase the DNA yield, use a higher volume of Elution Solution (KOA0912H). To increase the DNA concentration, use a lower volume of Elution Solution. In general, 150 µL is the recommended volume.

XII. RELATED PRODUCTS

General Use Magnetic Beads	Catalog #	Size (ml)
Trueblot® Anti-mouse IgG Magnetic beads (5 mg/ml)	00-1811-20 00-1811-50	2 5
Trueblot® Anti-rabbit IgG Magnetic beads (5 mg/ml)	00-1800-20 00-1800-50	2 5
Trueblot® Anti-Goat IgG Magnetic Beads (5mg/ml)	00-1844-20 00-1844-50	2 5
Trueblot® Streptavidin Magnetic Beads (5mg/ml)	S000-18-2 S000-18-5	2 5
Trueblot® Biotin Magnetic Beads (5 mg/ml)	B000-18-2 B000-18-5	2 5
Trueblot® Protein G Magnetic Beads (5mg/ml)	PG00-18-2 PG00-18-5	2 5
Trueblot® Nickel Magnetic beads (12.5 mg/ml)	N000-18-2 N000-18-5	2 5

Magnetic Bead IP/Purification Kits	Catalog #	Size (tests)
Trueblot® Protein G Magnetic Beads IP/Co-IP Kit	KBA-PG00-18	20
Trueblot® Protein A Magnetic Beads IP/Co-IP Kit	KBA-PA00-18	20

Magnetic Separators	Catalog #	Size (tests)
15/50 ml Tube Magnetic Separator	TMS-15-50	6/3
Multi-6 Microcentrifuge Tube Magnetic Separator	TMS-06	6
Multi-32 Microcentrifuge Tube Magnetic Separator	TMS-32	16