

RayBio Genomic DNA Magnetic Beads

Kit

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User Manual
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Caution:
Extraordinarily useful information enclosed



ISO 13485 Certified

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RayBiotech, Inc.

RayBio Genomic DNA Magnetic Beads Kit Protocol

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Please read the entire manual carefully before starting your experiment

I. General Description

RayBio's Genomic DNA Magnetic Purification 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 customized to optimize sample yield and quality depending on the type of sample. See Section 8 for customization options.

II. Safety Instructions

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

III. Storage and Stability

Upon delivery of the kit, remove the RNase A Solution and Proteinase K Solution vials and store at -20°C. Remove the DNA Purification Magnetic Beads and store at 4°C. **Do not freeze the magnetic beads solution.** All other kit reagents may be stored at room temperature (20-25°C). Do not use after the printed expiration date.

IV. How it Works

Sample Digestion: The crude sample, incubated with DNA Lysis Solution, Proteinase K Solution, and RNase A Solution to digest cells and denature proteins and RNA, is added to the magnetic beads.

Binding: DNA binds to the beads in the presence of the DNA Binding Solution. A magnet is used to secure the beads, with DNA attached.

Washing: Remaining cell debris is washed away in a series of two wash steps.

Elution: DNA is then eluted and transferred 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.

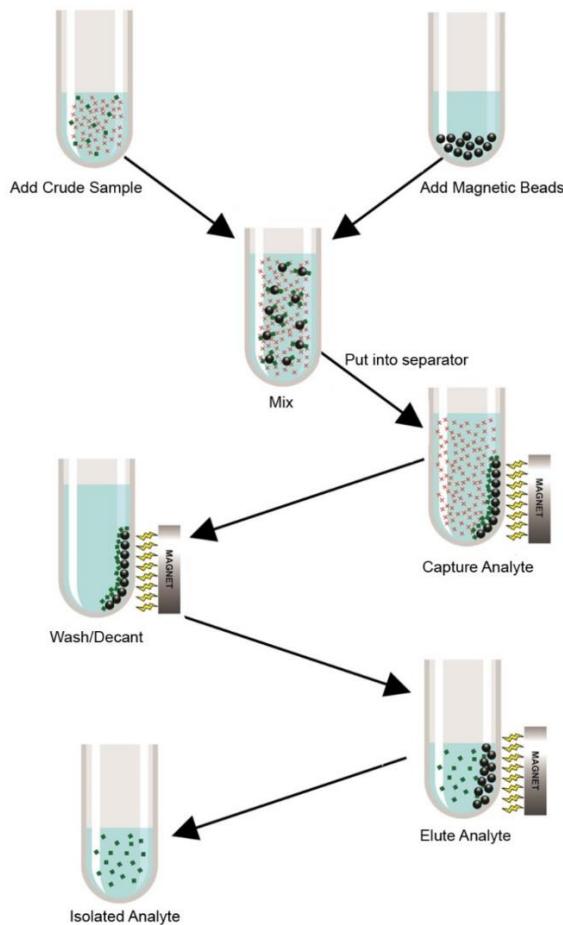


Figure 1. Schematic of RayBio Genomic DNA Magnetic Beads Kit process.

V. 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.

VI. Working Instructions

A. Materials Provided

1. DNA Lysis Solution, 20 mL
2. Proteinase K Solution, 2 mL
3. RNase A Solution, 2 mL
4. DNA Binding Solution concentrate, 20 mL
5. DNA Purification Magnetic Beads, 2 X 1 mL
6. DNA Wash Solution 1 concentrate, 14 mL
7. DNA Wash Solution 2 concentrate, 18 mL
8. DNA Elution Solution, 20 mL

B. Additional Materials Required

1. Disposable gloves and other protective equipment
2. Micro-pipettes with disposable plastic tips
3. 1.5 mL sterile plastic microcentrifuge tubes
4. 4°C refrigerator and -20°C freezer
5. 96-100% Ethanol
6. Tissue disruption equipment (dissection scissors, razor, mortar and pestle with liquid nitrogen, homogenizer etc.)
7. Balance
8. Vortexer
9. Heating block, thermomixer, or water bath capable of 65°C
10. Magnetic microcentrifuge tube separator, Solo (RayBio cat#: 801-206) or Multi-6 (RayBio cat#: 801-205) or similar
11. Minicentrifuge

12. Lysozyme buffer (Gram-positive bacteria only): 25 mM Tris-HCl pH 8.0, 2.5 mM EDTA, 1% Triton X-100, add fresh Lysozyme to 20 mg/mL concentration immediately before use.

C. Reagent Preparation

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

- 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

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

D. Procedures

1. Prepare Samples:

- Mammalian Tissues:
 1. 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.
 2. Weigh out up to 15 mg of tissue pieces.
 3. Collect tissue pieces into a 1.5 mL microcentrifuge tube (not provided) pre-filled with 200 μ L DNA Lysis Solution.
- Gram-negative Bacteria
 1. Add up to 2×10^9 Gram-negative bacterial cells (about 1 mL of overnight culture) to a 1.5 mL microcentrifuge tube (not provided).
 2. Centrifuge 10 minutes at 5,000 x g to pellet the cells, and discard the supernatant.
 3. Add 200 μ L DNA Lysis Solution and vortex or pipette up and down

to resuspend the pellet.

- Gram-positive Bacteria

1. Add up to 2×10^9 Gram-positive bacterial cells (about 1 mL of overnight culture) to a 1.5 mL microcentrifuge tube (not provided).
2. Centrifuge 10 minutes at $5,000 \times g$ to pellet the cells, and discard the supernatant.
3. Add 400 μL Lysozyme buffer with fresh Lysozyme (see Section 6.B.12) and vortex.
4. Incubate 1 hour at 37°C , vortexing occasionally.
5. Centrifuge 10 minutes at $5,000 \times g$ to pellet the cells, and discard the supernatant.
6. Add 200 μL DNA Lysis Solution and vortex or pipette up and down to resuspend the pellet.

2. Add 20 μL of Proteinase K Solution to the DNA Lysis Solution/sample tube and vortex well. Ensure that the sample is fully submerged in the solution mix.
3. 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.
4. Pulse spin (~1 second) the sample lysate with a minicentrifuge to remove any condensation from the sides and lid of the tube.
5. (*Optional*) Add 20 μL of RNase A Solution and vortex well. Incubate at room temperature for 10 minutes.
6. During the RNase A incubation, in a new 1.5 mL tube add 400 μL of 96-100% Ethanol (not provided). Vortex the DNA Purification Magnetic Beads well to ensure complete resuspension of the beads, and add 20 μL of beads to the ethanol. Vortex well.
7. After the RNase A incubation, add 300 μL of DNA Binding Solution (prepared with ethanol, see section 6.C) 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.
8. 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.

9. Remove the tube from the magnetic separator and add 500 μ L DNA Wash Solution 1 (prepared with ethanol, see section 6.C). 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.
10. Remove the tube from the magnetic separator and add 500 μ L DNA Wash Solution 2 (prepared with ethanol, see section 6.C). 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.
11. 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.
12. Remove the tube from the magnetic separator and add d 50-200 μ L DNA Elution Solution. Vortex briefly to resuspend the beads. See Section 8.B for elution volume recommendations.
13. Incubate the sample at 65°C for 10 minutes, vortexing occasionally or using a thermomixer.
14. Pulse spin to remove any condensation from the sides and lid of the tube.
15. 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.**

E. Storing DNA

Store the purified DNA in the DNA 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.

VII. Analyzing The Results

A. DNA Yield

DNA yield can be estimated by UV absorbance. Using a spectrophotometer blanked against the Elution Solution, measure the A_{260} (DNA absorbance) and A_{320} (turbidity/cuvette impurity) readings. Use the following equation:

$$\text{DNA yield (\mu g)} = (\text{A260 reading} - \text{A320 reading}) \times \text{dilution factor} \times 50 \text{ \mu g/mL} \times \text{sample elution volume (mL)}$$

Subtracting the absorbance at A_{320} results in a corrected reading that does not overestimate the DNA quantity. For DNA, an A_{260} of 1.0 = 50 μ g/mL when measured in a cuvette with a 10 mm optical path length.

Sample Type	Starting Amount	DNA Yield
Mouse brain	15 mg	Up to 9 μ g
Mouse ear chip	15 mg	Up to 14 μ g
Mouse lung	15 mg	Up to 22 μ g
Mouse muscle	15 mg	Up to 4 μ g
Mouse tail clip	15 mg	Up to 23 μ g
<i>E. coli</i> cells	$\sim 2 \times 10^9$ cells	Up to 15 μ g
<i>Lactobacillus</i> cells	$\sim 2 \times 10^9$ cells	Up to 13 μ g

Figure 2. Expected yield of genomic DNA from various samples types and starting quantities using the Genomic DNA Magnetic Beads Kit, standard protocol.

B. DNA Quality

Similarly, DNA quality can be estimated by UV absorbance readings. Measure the absorbance at A_{280} and A_{230} , and correct by subtracting the A_{320} absorbance. Highly pure DNA has an A_{260}/A_{280} ratio of ~ 1.7 – 2.0 , indicating it has minimal contamination by proteins, and an A_{260}/A_{230} ratio of >1.5 , indicating it has minimal contamination by organic compounds and salts. Below, Figure 3 displays a spectrophotometric curve of DNA purified from

mouse brain tissue, and Figure 4 shows genomic DNA from various samples analyzed by gel electrophoresis, another indicator of quality:

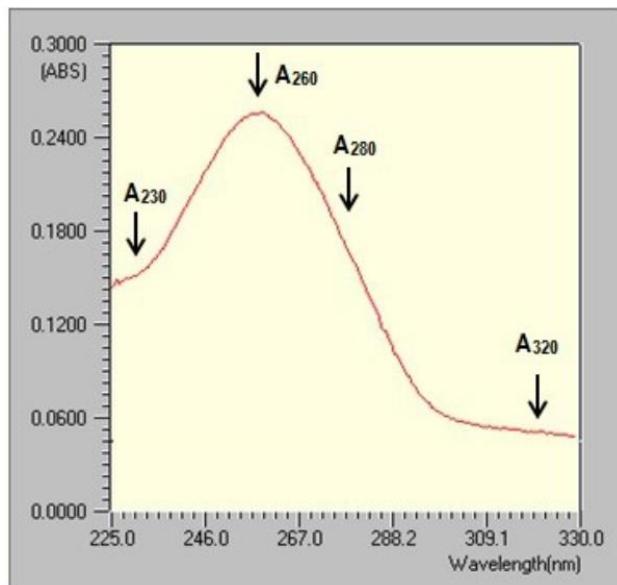


Figure 3. Spectrophotometric curve of high-purity DNA purified from 15 mg of mouse brain tissue using the Genomic DNA Magnetic Beads Kit standard protocol.

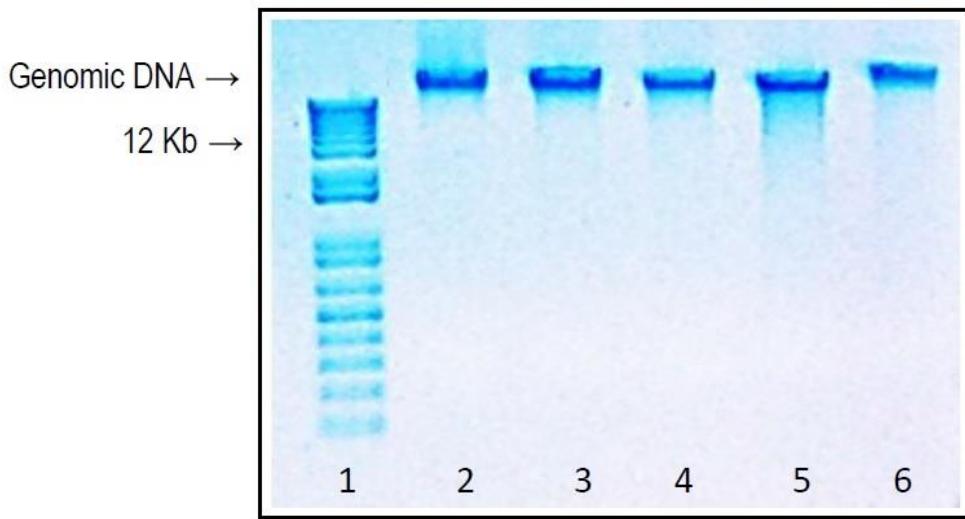


Figure 4. Agarose gel (1%) electrophoresis of 450 ng genomic DNA purified using the Genomic DNA Magnetic Beads Kit, standard protocol, for the following samples: Lane 1—1 Kb Plus molecular weight ladder, Lane 2—mouse brain, Lane 3—mouse ear, Lane 4—mouse lung, Lane 5—mouse tail, Lane 6—*E.coli* cells.

VIII. Protocol Customization Options

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 7.A). 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 quality and 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 6.D.1. Figure 5 below illustrates how increasing the starting sample amount increases the DNA yield:

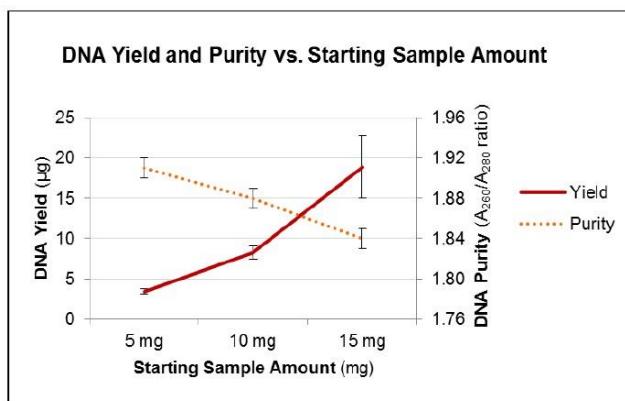


Figure 5. DNA yield (µg) vs. purity (corrected A260/A280 ratio – see Section 7.B) using starting amounts of 5, 10, or 15 mg of mouse tail tissue and 150 µL elution volume.

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 (see Section VII.A), 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 Solution Amount

Changing the amount of Elution Solution added in Section VI.D.12 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 6 below demonstrates the inverse relationship between concentration and yield:

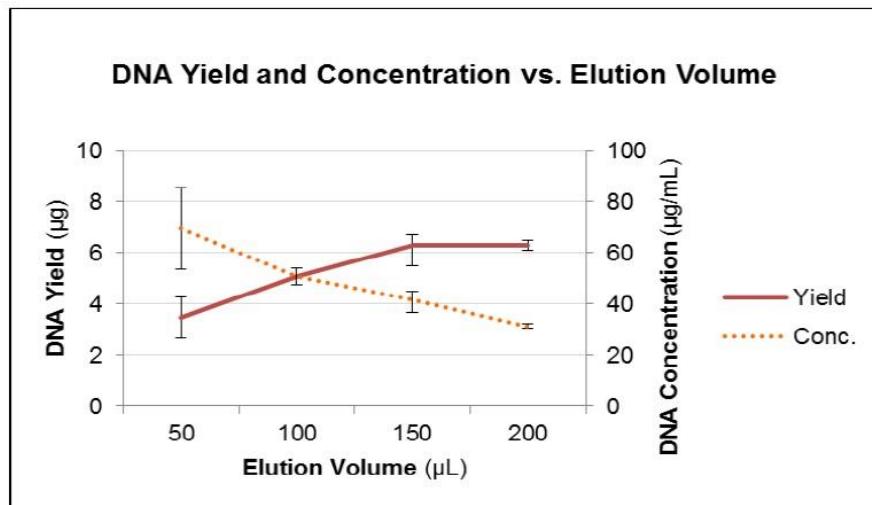


Figure 6. DNA yield (µg) vs. concentration (µg/mL) using $\sim 2 \times 10^9$ *E.coli* cells with increasing elution volumes.

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

This product is for research use only.



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