# DNA Purification Magnetic Beads

Catalog #: 801-109

User Manual

Last revised October 15th, 2020

Caution:

Extraordinarily useful information enclosed

RayBiotech

Empowering your proteomics

ISO 13485 Certified

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RayBiotech, Inc.

DNA Purification Magnetic Beads

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Please read the entire manual carefully before starting your experiment

## I. General Description

DNA Purification Magnetic Beads are 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

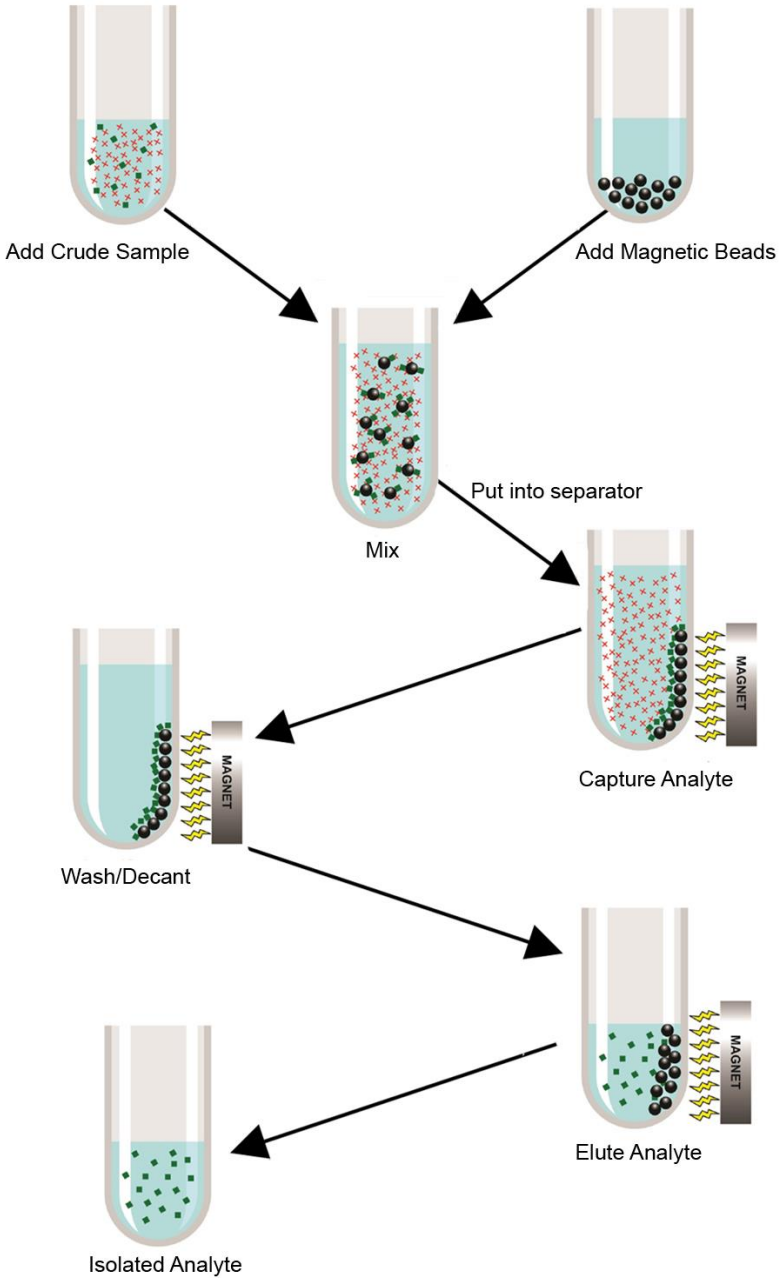
Use appropriate protective equipment (including but not limited to gloves, lab coats, and safety glasses) when collecting tissues and bacteria and using the kit. The DNA Binding Solution and DNA Wash Solution 1 contain guanidine hydrochloride, which can be irritating to eyes and skin. DO NOT ADD ACIDS OR BLEACH to liquid waste or spills containing guanidine hydrochloride, as contact with acids or bleach can release toxic gases! 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. Principle

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



## 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 Purification Magnetic Beads, 2 x 1 mL

### B. Additional Materials and Equipment Required

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 Wash Solution 1 concentrate, 14 mL
6. DNA Wash Solution 2 concentrate, 18 mL
7. DNA Elution Solution, 20 mL
8. Disposable gloves and other protective equipment
9. Micro-pipettes with disposable plastic aerosol barrier filter tips
10. 1.5 mL sterile plastic microcentrifuge tubes
11. 4°C refrigerator
12. -20°C freezer
13. 96-100% Ethanol
14. Tissue disruption equipment (dissection scissors, razor, mortar and pestle with liquid nitrogen, homogenizer etc.)
15. Balance
16. Vortexer
17. Heating block, thermomixer, or water bath capable of 65°C
18. Magnetic microcentrifuge tube separator, Solo (Product code: 801-206) or Multi-6 (Product code: 801-205) or similar

19. Minicentrifuge
20. 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).

- DNA Binding Solution: Add 12 mL 96-100% Ethanol
- DNA Wash Solution 1: Add 42 mL 96-100% Ethanol
- DNA 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. Procedure

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 µL DNA Lysis Solution.

- **Gram-negative Bacteria:**
    1. Add up to 2 x 109 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 µL DNA Lysis Solution and vortex or pipette up and down to resuspend the pellet.
- **Gram-positive Bacteria:**
    1. Add up to 2 x 109 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 x 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 x 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 bead solution 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 µL 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 µL 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 50-200 µL 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 Results

### A. DNA Yield

DNA yield can be estimated by UV absorbance. Using a spectrophotometer blanked against the DNA Elution Solution, measure the $A_{260}$ (DNA absorbance) reading. For DNA, an $A_{260}$ of 1.0 = 50 µg/mL when measured in a cuvette with a 10 mm optical path length. Use the following equation:

DNA yield (µg) = $A_{260}$ reading x dilution factor x 50 µg/mL x sample elution volume (mL)

In some cases, subtracting the turbidity/cuvette impurity absorbance at $A_{320}$ from the $A_{260}$ reading may be necessary for a corrected reading that does not overestimate the DNA quantity.

DNA yield can also be quantified using micro-fluorometers with specific intercalating dyes.

### 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 if necessary. 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.

## VIII. Protocol Customization

Many different types of mammalian tissues and bacteria may be used as starting material for DNA purification using the DNA Purification Magnetic Beads. 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.

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 7.A), decreasing the amount of starting material may be preferable if limited material is available, and/or to obtain higher purity ratios. 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. DNA Elution Solution Amount**

Changing the amount of DNA Elution Solution added in Section 6.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.

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