User Guide

PureBind Blood DNA Isolation Kit

For isolation and purification of genomic and mitochondrial DNA from human whole blood samples.

Version **190422**

Catalog **K0204-SMP**

K0204-100

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Product description

The PureBind Blood DNA Isolation Kit is designed to isolate DNA (genomic and mitochondrial) from human whole blood with high yield and purity. The kit utilizes automation-ready magnetic bead technology and reagents that enable efficient, consistent, and scalable isolation of DNA. Each standard prep uses 25 ul beads will typically yield about 10 ug of DNA from 350 ul of blood sample. The resulting DNA can be used in a wide range of downstream applications such as restriction enzyme digestion, PCR, qPCR, and NGS.

Contents and storage

Reagents have a one (1) year expiration date from date of purchase when stored properly. This kit contains beads and solutions sufficient for the specified number of isolations based on 350 ul of input blood sample.

PureBind Blood DNA Isolation Kit Catalog no.	4 preps [3] K0204-SMP	100 preps [3] K0204-100	Storage
PureBind Beads [1]	100 ul	3 ml	2-8°C
Lysis/Binding Buffer [1]	2 ml	40 ml	15-30°C
Wash Buffer 1 [1, 2] *Customer adds Isopropanol*	6 ml	2 x 60 ml	15-30°C
Wash Buffer 2 [1, 2] *Customer adds Ethanol*	3 ml	2 x 30 ml	15-30°C
Elution Buffer [1]	500 ul	10 ml	15-30°C

Note: Some reagents in each kit are provided in excess of the amount required.

[1] PureBind Beads are also available as Catalog no. A1602 and Lysis/Binding Buffer is available as Catalog no. R1247 and Wash Buffer 1 is available as Catalog no. R2340 and Wash Buffer 2 is available as R2355, and Elution Buffer is available as R0510.

[2] Prior to first time use, add the required amount of alcohol as indicated on the label of each bottle, then check the box and mix well.

[3] The number of preps or isolations are based on a 350 ul input blood sample.

Required materials not supplied

Item	Source	Catalog no.
Ethanol, absolute (100%)	Fisher Scientific	BP291914
Isopropanol (100%)	Sigma Aldrich	I9516
Proteinase K, lyophilized	Gold Biotech	P-480-100
Micro- and conical tubes (1-15 ml)	Various	Not applicable
Magnetic separator for micro-tubes	Ocean NanoTech	MMS
Vortex mixer	Various	Not applicable
Heat block for tubes	Various	Not applicable
Rotator (End-Over-End Mixer)	Thermo Fisher	HulaMixer
Pipettors (20-1000 ul) and tips	Various	Not applicable

Principle

The magnetic bead technology and reagents in this kit are used in a simple separation protocol to isolate DNA from human whole blood. A chemo-enzymatic lysis of white blood cells is followed by incubation with beads in the presence of isopropanol. During this incubation, the beads bind to DNA and are thereafter easily separated from the lysate using a magnet. Magnetic separation facilitates simple washing and elution of the isolated DNA.

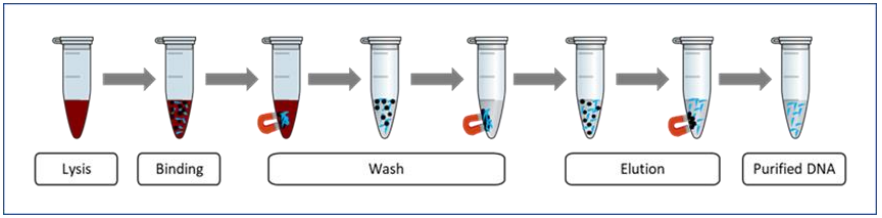

Figure. Illustration showing magnetic separation of DNA from human blood using the PureBind Blood DNA Isolation Kit.

Procedural guidelines

- Read the USER GUIDE and make sure all the directions are followed and all recommended volumes are used as indicated.
- Prior to use, bring all kit components to room temperature.
- All vortex steps should be performed at maximum speed to ensure mixing.
- Do not add proteinase K directly to the Lysis/Binding Buffer.
- PureBind Beads should be resuspended to a homogenous suspension prior to use.

Before you begin

Prepare working buffers

Prior to first time use, add isopropanol (100%) as indicated on the label of each bottle(s) of Wash Buffer 1 to obtain a working solution. Check the box on the label and mix well by inverting 10 times.

Prior to first time use, add ethanol (100%) as indicated on the label of each bottle(s) of Wash Buffer 2 to obtain a working solution. Check the box on the label and mix well by inverting 10 times.

Prepare proteinase K solution

Prepare enough proteinase K solution at 20 mg/ml (not provided) by dissolving lyophilized proteinase K powder in 50 mM Tris (pH 8.0). Then divide the stock solution into small aliquots and store at -20°C. Alternatively, a 20 mg/ml proteinase K solution can be purchased from Gold Bio (Catalog no. P-480-SL2) or a different supplier.

Protocol: Isolate genomic DNA from Human whole blood samples

The following protocol is for DNA isolation from 350 ul human whole blood. The protocol has been validated to scale up to a 10 ml and down to a 10 ul blood sample. If the amount of input blood sample between 10-350 ul, follow the procedures below without changing the amount of solutions. However, the amount of proteinase K, magnetic beads, lysis buffer, washing buffers, and elution buffer will need to be proportionally increased if the volume of input blood sample exceeds 350 ul.

Lyse sample and bind PureBind Beads to genomic DNA

1. Pipet 350 ul blood sample to a microtube.
2. Add 50 ul Proteinase K (20 mg/ml). Vortex for 30 seconds to mix. Then incubate for 2 minutes at room temperature.
3. Add 350 ul Lysis/Binding Buffer. Vortex for 30 seconds to mix. Then incubate for 10 minutes at 56°C followed by a final vortex for 30 seconds.
4. Add 25 ul PureBind Beads and 400 ul Isopropanol. Vortex for 30 seconds to mix. Then place the mixture in a rotator (end-over-end) for 10 minutes to bind genomic DNA to the beads, followed by a final vortex for 30 seconds.
5. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.

Wash PureBind Beads with wash buffers

6. Remove the tube from magnetic separator. Add 1 mL of Wash Buffer 1 and vortex for 30 seconds.
7. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.
8. Repeat Steps 6-7 for a second wash with the Wash Buffer 1.
9. Remove the tube from magnetic separator, add 1 mL of Wash Buffer 2 and vortex for 30 seconds.

10. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.
11. Repeat Steps 9-10 for a second wash with Wash Buffer 2.
12. Keep the tube on the magnetic separator with the lid open. Air dry the beads for 5 minutes. Remove any visible supernatant without disturbing the beads.

    Caution. Do not over dry beads as it may result in loss of cfDNA.

Elute the cfDNA

13. Remove the tube from magnetic separator and add 100 ul of Elution Buffer. Completely resuspend beads by pipetting 10 times. Then incubate for 2 minutes at room temperature.
14. Place the tube on the magnetic separator for 2 minutes or until the solution clears and beads are pelleted.
15. Carefully transfer the supernatant containing the purified genomic DNA without disturbing the pellet to a clean, labeled microcentrifuge tube.

    Note. The purified DNA is ready for immediate use. Alternatively, you can store the DNA at 4˚C for up to 24 hours or -20˚C for long-term storage.

Yield and quality measurements

DNA yield

We recommend using the Invitrogen™ Qubit™ dsDNA BR Assay Kit (Catalog no. Q32850 for total genomic DNA yield and quantification. This assay is specifically designed for use with the Qubit Fluorometer but can be used with any fluorometer or fluorescence plate reader.

DNA quality

We recommend using the NanoDrop or any spectrophotometer to determine the DNA quality. The NanoDrop is able to measure a sample using just 1 ul of sample and in less than five seconds to assess the purity of the sample such as proteins and nucleic acids.

Document History

Version	Person	Description of Change
150725	Luis Moreno	Initial draft
180716	Luis Moreno	Consultation draft – to Alice Bu
190422	Luis Moreno	Final version – approved by Luis Moreno

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Warranties and Disclaimers

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