4. Put the DW 96 deep well plate back into the instrument and continue the program. After about 26 minutes, the program finished running.
5. The DW 96 deep well plate and KF Duo elution strip were removed, and the DNA solution in the elution strip was transferred to a 1.5 mL centrifuge tube and stored at -20 °C.

Procedure (CW2361 matches KingFisher Flex)

After matching CW2361 with KingFisher Flex, blood genomic DNA could be extracted from 96 different blood samples with a volume of 200 uL.

1. Add the samples and reagents to the corresponding plates according to the table below:

Name	Type	Reagents and Dosage
Sample plate	DW 96 deep well plate	Proteinase K: 20 uL Blood: 200 uL Buffer ML: 200 uL
Wash plate I	DW 96 deep well plate	Buffer GW1: 700 uL KF 96 DW magnetic sleeve
Wash plate II	DW 96 deep well plate	Buffer GW1: 700 uL
Wash plate III	DW 96 deep well plate	Buffer GW2: 700 uL
Wash plate IV	DW 96 deep well plate	Buffer GW2: 700 uL
Elution plate	DW 96 deep well plate	Buffer EB: 100 uL

2. Start the software BindIt and import the CoWin Magbind Blood DNA Flex-200 program. The CoWin Magbind Blood DNA Flex-200 program was executed after placing the DW 96 deep-well plates with added reagents in sequence at the corresponding positions in the instrument.
3. After about 14 minutes, the instrument is stopped. Take out the sample plate and add 320 uL of thoroughly mixed Magbeads and isopropanol mixture to the sample plate.
4. Put the sample plate back into the instrument and continue the procedure. About 26 minutes later, the program was completed.
5. The eluting plate was removed, closed with film and stored at -20°C.

Version: 11/2022

Magbead Blood DNA Kit

Cat. No. : CW2361S (96 preps)
CW2361M (4×96 preps)

Shipping and Storage : Temperature (15-30°C)

Components

Component	CW2361S (96 preps)	CW2361M (4×96 preps)
Buffer ML	24 mL	96 mL
Buffer GW1(concentrate)	80 mL	4×80 mL
Buffer GW2(concentrate)	50 mL	4×50 mL
Buffer EB	30 mL	96 mL
Proteinase K	2×25 mg	180 mg
Proteinase K Storage Buffer	2×1.25 mL	2×5 mL
Magbeads PN	2×1 mL	8 mL

Principle

The kit provides a simple, rapid, and efficient method for blood DNA extraction from fresh or frozen anticoagulant blood (citrate, EDTA, heparin treated blood samples). In the presence of high salt, DNA binds to the surface of the silicon-based coated Magbeads. After rinsing, the highly purified DNA is eluted in BufferEB or deionized water. DNA yield depends on the type of sample, storage conditions, time, and the content of white blood cells in the sample. 7-10 μg genomic DNA can usually be extracted from 200 uL of cryopreserved anticoagulant blood. The purified DNA has good purity (A260/280 ratio between 1.8-2.0, A260/230 ratio more than 1.5-2.5) and high integrity (up to 150 kb), which can be used for next-generation sequencing, cloning, quantitative PCR, chip detection and other downstream experiments.

The kit can be used with liquid workstation and magnetic rod method automatic magnetic bead extraction system, simple and rapid large-scale extraction, greatly reducing the experimenter's workload and human error in the experiment.

Reagents to Be Supplied by User

1. Manual single tube extraction:
   1）Constant temperature mixer -- Cat. No. : CW2593
   2）2/15mL magnetic stand -- Cat. No. : CW259
   3）isopropyl alcohol, absolute ethanol
2. Manual extraction of 96-well deep well plate:
   1）Constant temperature mixer -- Cat. No. : CW2593
   2）Waste liquid suction system – Cat. No. : CW2616
   3）Manual continuous liquid dispenser -- recommend brand Eppendorf
   4）Electric continuous liquid separator -- recommend brand Eppendorf
   5）Manual continuous dispenser dispenser tube (25 mL) -- recommend brand Eppendorf
   6）96-well plate magnetic bracket – Cat. No. : CW2595
   7）isopropyl alcohol, absolute ethanol
3. Magnetic bead automatic extraction system by magnetic bar method:
   1）Magnetic bead automatic extraction system by magnetic rod method -- Thermo Fisher is recommended
   2）isopropyl alcohol, absolute ethanol

Important Points Before Starting

1. Add the specified amount of Proteinase K Storage Buffer to Proteinase K to dissolve it and save it at -20°C.
   Proteinase K prepared should not be placed at room temperature for a long time to avoid repeated freezing and thawing, so as not to affect its activity.

Proteinase K	25 mg	180 mg
Proteinase K Storage Buffer	1.25 mL	9 mL

15. Add 100-200 uL Buffer EB to the deep well plate with an electric continuous liquid separator or 8-channel pipette, and then put the deep well plate on a constant temperature mixer at 100°C (as the constant temperature mixer is heated in suspension, the actual temperature of the eluent is between 50-60°C) and 1600 rpm for 10 minutes.
16. The deep-well plate was removed from the constant temperature mixer and placed on the magnetic rack of the 96-well plate for 2 minutes. The solution was transferred to the 96-well PCR plate with an 8-channel pipette solution and then covered and stored at -20°C for later use.

Procedure (Matching CW2361 with KingFisher Duo)

CW2361 was matched with KingFisher Duo to extract genomes from 12 different blood samples with a volume of 200 uL DNA.

1. Add the samples and reagents to the corresponding positions according to the table below:

Name	Position	Reagents and Dosage
DW 96 deep well plate	A1-A12	Proteinase K: 20 uL Blood: 200 uL Buffer ML: 200 uL
	B1-B12	KF Duo 12-channel magnetic sleeve
	C1-C12	Buffer GW1: 700 uL
	D1-D12	Buffer GW1: 700 uL
	E1-E12	Buffer GW2: 700 uL
	F1-F12	Buffer GW2: 700 uL
A1-A12	KF Duo elution strip	Buffer EB: 100 uL

2. Start the software BindIt and import the CoWin Magbind Blood DNA Duo-200 program. CoWin Magbind Blood DNA DUO-200 was performed after the DW 96 deep well plate and KF Duo elution strip with samples and reagents were placed into the KingFisher Duo instrument.
3. After about 12 minutes, the instrument was stopped. The DW 96 deep well plate was removed and 320 uL of thoroughly mixed Magbeads and isopropanol mixture was added to the A1-A12 Wells.

5. The deep-well plate was removed from the constant temperature mixer and placed on the 96-well plate magnetic rack for 2 minutes. Discard solution with waste suction system or 8-channel pipette, avoiding contact with magnetic beads during the process.

   Note: When removing solution with waste liquid suction system, the vacuum pump should be adjusted to a small negative pressure value, so that the solution can be sucked away at a suitable speed, too fast speed will cause the loss of magnetic beads.
6. A manual continuous liquid separator was used to add 500 uL Buffer GW1 to the deep well plate (check whether absolute ethanol had been added before adding), and then the deep well plate was fixed on a constant temperature mixer at 25°C and 1600 rpm for 3 minutes.
7. The deep-well plate was removed from the constant temperature mixer and placed on the 96-well plate magnetic rack for 2 minutes. Discard solution with waste suction system or 8-channel pipette, avoiding contact with magnetic beads during the process.
8. Repeat steps 6-7.
9. A manual continuous liquid separator was used to add 500 uL Buffer GW2 to the deep well plate (check whether absolute ethanol had been added before adding), and then the deep well plate was fixed on a constant temperature mixer at 25°C and 1600 rpm for 3 minutes.
10. The deep-well plate was removed from the constant temperature mixer and placed on the 96-well plate magnetic rack for 2 minutes. Discard solution with waste suction system or 8-channel pipette, avoiding contact with magnetic beads during the process.
11. Repeat steps 9-10.
12. A manual continuous liquid separator was used to add 500 uL of absolute ethanol into a 96-well deep well plate, and then the deep well plate was fixed on a constant temperature mixer at 25°C at 1600 rpm for one minute.
13. The plate was removed from the constant temperature mixer and placed on the 96-well plate magnetic rack for 2 minutes. Discard liquid with waste suction system or 8-channel pipette, avoiding contact with magnetic beads during the process.
14. Keep the deep-well plate fixed on the magnetic rack of the 96-well plate, and place the deep-well plate upside down on clean absorbent paper for 2 minutes. Then, the deep-well plate was removed from the 96-well plate magnetic frame and placed on a constant temperature mixer at 100°C and 1600 rpm for 5 minutes.

2. Preparation of mixture of isopropyl alcohol and Magbeads (Take the amount required for preparation and extraction of 10 samples as an example)

   1）Add 3.3 mL [0.3× (10+1) =3.3] isopropyl alcohol to centrifuge tubes with appropriate capacity (the total volume after adding isopropyl alcohol and Magbeads is less than 2/3 of the centrifuge tube volume).

   Note: If the pipette is used to add isopropyl alcohol, the pipette should be used to blow and suck isopropyl alcohol twice and then slowly absorb isopropyl alcohol.

   2）Add 220 uL [20×(10+1)=220] Magbeads into the centrifuge tube at the next step.

   Note: Before adding Magbeads, swirl for 20 seconds to mix thoroughly. Swirl before using the mixture of isopropyl alcohol and Magbeads Shake for 20 seconds to make a uniform solution.

   3）The mixture of isopropyl alcohol and Magbeads needs to be vortexed for 10 seconds before use to make it a uniform solution.
3. Magbeads are strictly prohibited from freezing and centrifuging. Freezing and centrifugation may cause irreversible damage to Magbeads.
4. Repeated freezing and thawing of the sample should be avoided, otherwise the extracted DNA fragments will be small and the extraction rate is low.
5. Before use, add absolute ethanol to Buffer GW1 and Buffer GW2 according to the label of the reagent bottle.
6. If precipitate appears in Buffer ML, redissolve it in a 56℃ water bath and shake well before use.
7. During the experiment, the full mixing of magnetic beads in the solution has a great influence on the yield and purity of the extraction. The magnetic beads must be thoroughly mixed with the solution during the experiment. The oscillatory mixing effect of the constant temperature mixer produced by different manufacturers is different. Please pay attention to the state of magnetic beads during the experiment. If the magnetic beads stick to the wall and other insufficient mixing phenomenon, please use the pipette to blow and suck the mixing or adjust the vibration frequency.

Procedure (Manual single-tube operation)

1. Add 20 uL Proteinase K to a 1.5 mL centrifuge tube, followed by 200 uL of blood.
   Note: 1) Frozen anticoagulant blood should be placed at room temperature (15-30℃) in advance, melted and mixed.
   2) If the blood volume is greater than or less than 200 uL, the dosage of ProteinaseK, Buffer ML and the mixture of isopropanol and magnetic beads should be adjusted according to the proportion.
2. Add 200 uL Buffer ML to the centrifuge tube, swirl and oscillate for 5 seconds to fully mix it, then place the centrifuge tube in a water bath at 56°C and incubate for 15 minutes, during which vortex and oscillate for 2 times.
3. Remove the tube from the water bath, centrifuge briefly and let stand at room temperature for 5 minutes. Add 320 uL of thoroughly mixed isopropyl alcohol and magbeads mixture. Swirl and mix for 5 seconds. Then place the centrifuge tube on a constant temperature mixer at 25°C and 1600 rpm for 5 minutes or invert the centrifuge tube for 10 minutes.
4. Place the centrifuge tube on the magnetic stand for 1 minute. After magbeads are absorbed into the side wall of the centrifuge tube, discard the solution completely (keep the tube fixed on the magnetic stand).
5. Remove the centrifuge tube from the magnetic stand, add 750 uL Buffer GW1 (please check whether absolute ethanol has been added before use), swirl for 1 minute or swirl for 5 seconds, and then place on a constant temperature mixer at 25°C and 1600 rpm for 2 minutes. (Ensure that magbeads are in the mixing state during the shaking process.) Then place the centrifuge tube on the magnetic rack for 1 minute. After Magbeads are completely adsorbed on the side wall of the centrifuge tube, gently reverse the magnetic bead, wash the impurities on the cover of the centrifuge tube and discard the solution completely (keep the centrifuge tube fixed on the magnetic rack).
6. Repeat Step 5.
7. Remove the centrifuge tube from the magnetic stand, add 750 uL Buffer GW2 (please check whether absolute ethanol has been added before use), swirl for 1 minute or swirl for 5 seconds, and then place on a constant temperature mixer at 25°C and 1600 rpm for 2 minutes. (Ensure that magbeads are in the mixing state during the shaking process.) Then place the centrifuge tube on the magnetic rack for 1 minute. After magbeads are completely adsorbed on the side wall of the centrifuge tube, gently reverse the magnetic bead, wash the impurities on the cover of the centrifuge tube and discard the solution completely (keep the centrifuge tube fixed on the magnetic rack).

8. Repeat Step 7.
9. Keep the centrifuge tube in place on the magnetic rack, and use a pipette to further remove the solution from the bottom and cover of the tube, then leave it at room temperature for 5-10 minutes to allow the ethanol to evaporate.
   Note: If there are liquid beads on the side wall of the centrifuge tube, add 750 uL absolute ethanol to the tube. Cover and reverse the centrifuge tube (keep the tube fixed on the magnetic mount), then discard the absolute ethanol completely.
10. Remove the centrifuge tube from the magnetic rack and add 50-200 uL Buffer EB. The magnetic beads were completely suspended in the eluent by vortex oscillation and then placed on a constant temperature mixer at 56°C and 1600 rpm to oscillate and eluate for 10 minutes, or the centrifuge tube was placed in a water bath at 56°C and incubated for 10 minutes, during which the vortex was oscillated for 10 seconds every 3 minutes.
11. Place the centrifuge tube on the magnetic stand for 2 minutes. After magbeads are completely adsorbed on the side wall of the centrifuge tube, transfer the eluent to a new centrifuge tube with a pipette and store at -20°C for later use.

Operation Procedure (manual operation with 96-well deep hole plate)

1. 20uL Proteinase K was added to 2mL 96-well deep well plate (referred to as "deep well plate"), followed by 200uL blood, and the name of blood added to each well was recorded.
   Note: 1) Proteinase K can be pre-packed in 8 tubes, adding 260 uL to each tube. After that, Proteinase K was divided into 96-well deep well plates with an 8-channel pipette.
   2) Blood should be added to the bottom of the 96-well deep well plate to avoid blood touching the upper part of the hole.
2. Add 200 uL Buffer ML to the deep well plate using a motorized continuous liquid divider or 8-channel pipette.
3. The deep-well plate was fixed on a constant temperature mixer at 25°C and 1600 rpm for shaking and mixing for 2 minutes. After the silicone cap was covered, the deep-well plate was placed in a water bath at 56°C and incubated for 15 minutes, and then the deep-well plate was fixed on a constant temperature mixer at 25°C and 1600 rpm for shaking and mixing for 5 minutes.
4. Remove the deep well plate from the constant temperature mixer and add 320 uL of thoroughly mixed Magbeads and isopropanol mixture into the deep well plate using an electric continuous liquid separator or an 8-channel pipette. After the silicone cap was covered, the deep-well plate was fixed on the constant temperature mixer at 25°C and 1600 rpm for 5 minutes by shaking.