

Hieff™ Blood DNA Kit

Product description

The Hieff™ Blood DNA Kit is designed for the extraction of DNA from blood samples of mammals, poultry, birds, amphibians, and other species.

The spin columns utilize a unique silica-based matrix material proprietary to our company. When combined with our specialized lysis buffer formulation, this system enables the maximum recovery of high-purity DNA.

The extracted DNA exhibits high purity and consistent quality, making it suitable for various downstream applications such as PCR, molecular cloning, and restriction enzyme digestion.

Specifications

Cat.No.	18730ES08 / 18730ES50 / 18730ES70
Size	5 T / 50 T / 200 T

Components

Components No.		Name	18730ES08 (5 T)	18730ES50 (50 T)	18730ES70 (200 T)
PartI	18730-A	ProteinaseK(20mg/ mL)	100 µL	1 mL	1 mL×4
	18730-B	Hieff™ DNA Column B2	5	50	200
PartII	18730-C	2 mL Collection Tube B2	5	50	200
	18730-D	AC Buffer B2	500 µL	5 mL	20 mL
	18730-E	DB Buffer B2	1 mL	10 mL	40 mL
	18730-F	Binding Buffer (BD Buffer B2)	1.5 mL	15 mL	60 mL
	18730-G	Protein Remove Buffer*(PL Buffer B2*)	1.6 mL	16 mL	64 mL
	18730-H	Wash Buffer*	1.5 mL	13 mL	50 mL
	18730-I	Elution Buffer	1 mL	10 mL	20 mL

Storage

Store Part I components at 2–8 °C for 2 years, protected from light.

Store Part II components at room temperature. Shelf life: 2 years.

Notes

1. To prevent evaporation, oxidation, and pH changes caused by prolonged exposure to air, promptly close the caps of all solutions after use.
2. Check for precipitation or turbidity in the solutions (especially at low room temperatures, such as during winter).
If observed, incubate at 37°C until the solution becomes clear again to ensure optimal performance.

3. The Protein Removal Buffer (PL) contains irritants. For your safety and health, please wear a lab coat and disposable gloves when handling this reagent.
4. For Research Use Only!

Materials Preparation

1. Supplied by User: Tabletop centrifuge, vortex mixer, water bath or dry bath, isopropanol, microcentrifuge tubes, etc.
2. Unless otherwise specified, all centrifugation steps should be performed at room temperature using a conventional tabletop centrifuge capable of reaching 12,000 rpm.

3. Before the first use, **Protein Removal Buffer PL (18730-G)**:

Add absolute ethanol to Protein Removal Buffer PL at a ratio of 1.6:1 (PL*:ethanol). For example:

Add 1 mL of absolute ethanol to 1.6 mL of Protein Removal Buffer PL*.

Add 10 mL of absolute ethanol to 16 mL of Protein Removal Buffer PL*.

Add 40 mL of absolute ethanol to 64 mL of Protein Removal Buffer PL*.

Wash Buffer W (18730-H):

Add 4 volumes of absolute ethanol to Wash Buffer W (e.g., add 40 mL of absolute ethanol to 10 mL of Wash Buffer W*). Mix thoroughly before use and clearly label the bottle. If the volume of Wash Buffer W* has changed significantly due to improper transport or storage, please adjust to the correct volume using a graduated cylinder before adding the 4 volumes of absolute ethanol. After each use, tightly close the cap to maintain the ethanol concentration in Wash Buffer W*.

Instructions

1. Sample Pretreatment

- 1) Take 200 µL of fresh, frozen, or anticoagulated blood and transfer it to a 1.5 mL microcentrifuge tube.

[Note]: If the volume is less than 200 µL, adjust it to 200 µL with Buffer DB. PBS or normal saline can also be used as a substitute for Buffer DB.

[Note]: If the processing volume is between 200~300 µL, adjust the reagent volumes in subsequent steps proportionally.

[Note]: If the processing volume is between 300 µL and 1 mL, treat the sample with a red blood cell (RBC) lysis buffer.

[Note]: For birds, poultry, and other species with nucleated red blood cells, it is recommended to use 5~20 µL of blood and adjust the volume to 200 µL with Buffer DB.

[Note]: Fresh blood or specimens stored at 2~8°C for less than 3 days are recommended. Avoid using specimens that have undergone more than three freeze-thaw cycles, as this will significantly reduce the yield.

- 2) Add 20 µL of **Proteinase K (20 mg/mL)** and mix thoroughly.

- 3) (Optional) If residual RNA may interfere with downstream experiments, add 5 µL of **RNase A solution (100 mg/mL, supplied by user, Cat# 10406ES)**, vortex to mix, and incubate at room temperature for 5~10 min.

- 4) Add 200 µL of **Binding Buffer BD**, vortex immediately to mix, and briefly centrifuge to collect the liquid from the tube walls.

- 5) Incubate at 70°C in a water bath or dry bath for 10 min.

- 6) After cooling, add 100 µL of isopropanol (supplied by user) and vortex vigorously for 15 sec to mix.

[Note]: Flocculent precipitation may form; this is normal.

2. DNA Purification

- 1) Insert DNA Adsorption Column B2 into a 2 mL collection tube and set aside.
- 2) Add **100 µL of Buffer AC** to the DNA Adsorption Column B2, centrifuge at 12,000 rpm for 1 min, and discard the flow-through.
- 3) Transfer the pretreated mixture to the DNA Adsorption Column B2, centrifuge at 12,000 rpm for 1 min, and discard the flow-through.
- 4) Add 500 µL of **Protein Removal Buffer PL**, centrifuge at 12,000 rpm for 30 sec, and discard the flow-through.
- 5) Place the DNA Adsorption Column B2 back into the collection tube, add 600 µL of **Wash Buffer W***, centrifuge at 12,000 rpm at room temperature for 30 sec, and discard the flow-through.

[Note]: Ensure that ethanol has been added to the Wash Buffer W*.

- 6) Repeat step 5.
- 7) Place the DNA Adsorption Column B2 back into the collection tube, centrifuge at 12,000 rpm at room temperature for 2 min to remove residual Wash Buffer W*.
- 8) Transfer the DNA Adsorption Column B2 to a new 1.5 mL microcentrifuge tube. Add 50~100 µL of **Elution Buffer** to the center of the column, incubate at room temperature for 2 min, and then centrifuge at 12,000 rpm for 1 min. The collected filtrate is the purified DNA solution.

[Note]: The yield can be improved by:

- a. Pre-heating the Elution Buffer to 70°C.
- B. Re-applying the eluate to the column, incubating at room temperature for 2 min, and eluting again.

[Note]: The number of white blood cells varies greatly among different samples, especially disease samples, which may result in significant individual variations in yield.

- 9) The DNA solution can be stored at -80°C for long-term preservation.

Appendix: Red Blood Cell (RBC) Lysis Procedure

- 1) Transfer 3 mL of Red Blood Cell Lysis Buffer (supplied by user, Cat# 40401ES) to a 15 mL centrifuge tube.

[Note]: It is recommended to collect blood samples using anticoagulants other than heparin.

- 2) Add 1 mL of anticoagulated whole blood (warmed to room temperature) and invert the tube 6~8 times. Gently tap the tube wall upside down to ensure thorough mixing.
- 3) Incubate at room temperature for 5~10 min. Invert and mix several times every 2~3 minutes to aid in RBC lysis.
- 4) Centrifuge at 3,000 rpm at room temperature for 5 min and carefully discard the supernatant.

[Note]: A white pellet of white blood cells should be visible at the bottom of the tube. If a large red cell pellet remains, the RBC lysis was insufficient. Add more lysis buffer, resuspend the pellet, and repeat steps 3 and 4.

- 5) Add 200 µL of Buffer DB and vortex to resuspend the white blood cell pellet, ensuring it is fully dispersed.
- 6) Proceed with DNA extraction following the Sample Pretreatment and DNA Purification steps outlined above.