

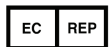
Free-Circulating DNA Kit Instructions for Use

For In-vitro Diagnostic (IVD) Use Only

Basic Information



Jiangsu CoWin Biotech Co., Ltd.
No.18, Zelan Road, China Medical City, Taizhou, Jiangsu, China.
Manufacturing address:
No.18, Zelan Road, China Medical City, Taizhou, Jiangsu, China.
Post code: 225300
Tel: +86-523-86201352 Fax: +86-523-86816890
Website: www.cwbiosciences.com
After-sales service Tel: +86-10-56953015
E-mail: info@cwbio.com



CMC Medical Devices & Drugs S.L
Address: C/Horacio Lengo N° 18 CP 29006, Málaga-Spain
Tel: +34 951 214054 Email: info@cmcmedicaldevices.com

For purification of DNA from: plasma, serum, urine, lymph, cerebrospinal fluid, amniotic fluid, hydrothorax and ascites, or other cell-free body fluids.

Version: A/0 Effective Date: 2024-10-23

Name/Trade name

Free-Circulating DNA Kit

Packaging Specifications

Type VIII: 48 tests/box

Intended Use

It is used for nucleic acids extraction, enrichment, purification and other steps. The processed products can be used for clinical diagnosis in vitro.

Test Principle

After samples lysis, in the presence of high salt, DNA binds to the surface of silicon-coated magbeads, and after rinsing, high-purity DNA is eluted in elution buffer or deionized water. The yield of DNA is closely related to the type of samples, storage conditions, time and individual differences.

Components of the Kit

Type VIII: MagBead High-Yield Max Volume Method

Table 1 Components of Type VIII

Reagent name	Specifications (48 tests/box)
Lysis Buffer 1	120 mL/bottle × 1
Lysis Buffer 2	6 mL/bottle × 1
Washing Buffer 1 (concentrate)	80 mL/bottle × 1
Washing Buffer 2 (concentrate)	40 mL/bottle × 1
Elution Buffer	30 mL/bottle × 1
Proteinase K	1.25 mL/tube × 2
Magbeads Suspension Solution	1 mL/tube × 2
Carrier RNA	310 µg/tube × 1

Warnings and Precautions

1. For in vitro diagnostic use (IVD) only.
2. This product is intended for professional use only.
3. Check the package before use it. If damaged, it is strictly prohibited to use.
4. DO NOT drink, touch or remove the reagent from the vial.
5. Avoid reuse.
6. It is forbidden to use expired products, and do not mix different reagents.
7. If any serious incident that has occurred in relation to the product shall be reported to the manufacturer and the competent authority.
8. This kit is intended to be used with verification equipment, such as applicable equipment mentioned in the instructions for use. If you want to use with other equipment, please verify equipment first.
9. Read the Instructions for Use carefully before use.
10. Dispose of as hazardous waste in compliance with local and national regulations.
11. Used products maybe contain pathogens or infectious agents, that will cause infection. Used product and product that are past their shelf-life must be disposed of as special waste in accordance with local guidelines.

Glossary of Symbols

Symbol	Meaning	Symbol	Meaning
	<i>In vitro</i> diagnostic medical device		Temperature limit
	Manufacturer		Use-by date
	Date of Manufacture		Do not re-use
	Batch code		Consult instructions for use
	Authorized representative		CE Symbol
	Caution		Part Number
	Catalogue number		Do not use if package is damaged and consult

- 1.13. Add 50–100 μL of elution buffer into the centrifuge tube, use a pipettor to make the magbeads fully suspended in the elution buffer, then fix the centrifuge tube on a constant temperature mixer at 25°C and 1600 rpm for shaking elution for 10 minutes.
- 1.14. Fix the centrifuge tube on the magnetic device and let it stand for 2 minutes. After the magbeads are completely adsorbed on the side wall of the centrifuge tube, use a pipettor to transfer the eluent to a new centrifuge tube for storage at $-20\pm 5^\circ\text{C}$.

2. Matching with CWE240 (take 2mL of samples as the example)

After the product is matched with CWE240, DNA can be extracted from 1–24 body fluid samples at one time.

2.1. Add reagents to the 24 deep well plate as shown in the following table

Table 4 The added reagent and volume

Position of CWE240	Reagent and volume
1	Proteinase K: 30 μL Samples: 2 mL Lysis Buffer 2: 100 μL Carrier RNA: 5 μL
2	Washing Buffer 1: 1 mL
3	Washing Buffer 1: 1 mL
4	Washing Buffer 2: 1 mL
5	Washing Buffer 2: 1 mL
6	Elution Buffer: 65–100 μL

- 2.2 Place the 24 channel tip comb and 24 deep well plate into the corresponding positions of CWE240 nucleic acid extractor, and run the extraction program.
- 2.3. After about 23 minutes, the instrument is suspended, take out the deep well plate 1 from the instrument and immediately place it on ice for 5–10 minutes. Then, add 2 mL of lysis buffer 1, 0.5 mL of isopropanol and 30 μL of magbeads suspension solution to each well of the deep well plate 1.
- 2.4. Place the deep well plate 1 back into the instrument and continue to run the program. After about 45 minutes, the program ends. Transfer the elution product of free DNA in deep well plate 6 to a centrifuge tube, storage at $-20\pm 5^\circ\text{C}$.

Storage Condition and Shelf Life

The Magbeads Suspension Solution and Carrier RNA are stored at 2–8°C, and the other components are stored at 4–30°C, and the validity period is 15 months.

It can be transported at 4–37°C, and the transportation time is suggested no more than 30 days.

Specimen Requirements

1. Applicable specimen types: Plasma, serum, urine, lymph, cerebrospinal fluid, amniotic fluid hydrothorax and ascites or other cell-free body fluids.
2. Specimen processing and storage: Serum and plasma samples are prepared by conventional methods, and fresh samples should be treated as soon as possible or frozen at -70°C after subpackage, avoid repeated freezing and thawing.

Materials and Equipment Required but Not Provided

Type VIII: MagBead High-Yield Max Volume Method

1. Manual single-tube extraction:
 - (1) Constant Temperature Mixer, – recommended brand: CWBIO, catalog: CW2593
 - (2) 2/15 mL Magnetic Device, – recommended brand: CWBIO, catalog: CW2594
 - (3) Ethanol and isopropanol
2. Matching with CWBIO CWE240 Automatic Nucleic Acid Extractor:
 - (1) Automatic Nucleic Acid Extractor – recommended brand: CWBIO, CWE240
 - (2) Ethanol and isopropanol
 - (3) 24 Deep Well Plate and 24 Channel Tip Comb, – recommended brand: CWBIO, catalog: CW3075S

Precautions Before Use

1. Before the first use, add the required reagent according to the bottle label.
2. Check if there is crystallization or precipitation in the reagent. If there is crystallization or precipitation, please dissolve the reagent again in a 56°C water or metal bath, mix well, and then leave it to room temperature for use.
3. DO NOT repeat freezing and thawing samples, otherwise the extracted DNA fragments will be small and the extraction yield will be low.

4. DO NOT freeze and centrifuge the magbeads suspension solution, otherwise it may cause irreversible damage to the magbeads.
5. Before using the magbeads suspension solution, vortex it for 20 seconds to make it fully mixed.
6. Dissolving Carrier RNA (0.2μg/μl): Add 1550 μl of Elution Buffer to the tube containing the Carrier RNA powder to achieve a final concentration of 0.2μg/μl. Vortex to fully dissolve the Carrier RNA powder. After dissolving, aliquot the solution and store it at -20±5°C. Do not exceed three freeze-thaw cycles for the dissolved Carrier RNA. Carrier RNA can improve the recovery efficiency of trace nucleic acids when the nucleic acid content in the sample is very low.

Test Method

Type VIII: MagBead High-Yield Max Volume Method

This type can extract 48 times for 2 mL of samples; 24 times for 4 mL of samples; 96 times for 1 mL of samples.

1. Manual single-tube detection (take 2 mL of samples as the example)
 - 1.1. Add 30 μL of proteinase K solution, 2 mL of samples, 100 μL of lysis buffer 2 and 5 μL Carrier RNA(1μg) to the centrifuge tube in turn according to the following table, put them on a constant temperature mixer at 60°C and 1200 rpm, and shake them for 20 minutes. After incubation, ice bath the centrifuge tube for 5-10 minutes.

Note: (1) If there is no constant temperature mixer, vortex the centrifuge tube for 10 seconds then put it in a 60°C water bath or metal bath for 20 minutes, and vortex it for 10 seconds every 7 minutes.

(2) To avoid inactivation of proteinase K, please add the reagents in the following order, and do not add lysis buffer 2 directly into proteinase K solution.

Table 2 Order of adding different component

Component	Volume of samples			
	1 mL	2 mL	4 mL	10 mL
Proteinase K solution	15 μL	30 μL	60 μL	150 μL
Samples	1 mL	2 mL	4 mL	10 mL
Lysis buffer 2	50 μL	100 μL	200 μL	500 μL
Carrier RNA	5 μL	5 μL	5 μL	10 μL
Total volume	1.07 mL	2.135 mL	4.265 mL	10.66 mL

- 1.2 During incubation, prepare the lysis buffer/magbeads suspension solution according to the following table and mix evenly.

Table 3 Order of adding different component

Component	Volume of samples			
	1 mL	2 mL	4 mL	10 mL
Lysis buffer 1	1 mL	2 mL	4 mL	10 mL
Isopropanol	0.25 mL	0.5 mL	1 mL	2.5 mL
Magbeads Suspension Solution	15 μL	30 μL	60 μL	150 μL
Total volume	1.265 mL	2.53 mL	5.06 mL	12.65 mL

- 1.3. Add the lysis buffer/magbeads suspension solution prepared in step 1.2 to the sample tube in step 1.1. Vortex for 1 minute, then manually invert or use a mixer to mix for 5-10 minutes, keeping the magbeads in a suspended state.
- 1.4. Place the centrifuge tube on a magnetic device and let it stand. After the magbeads are adsorbed onto the magnetic device and the solution inside the tube becomes clear, turn the centrifuge tube over to wash the residual magbeads on the bottle cap, leave it for about 1 minute, and then discard the solution.
- 1.5. Add 1 mL of washing buffer 1 (please check whether ethanol has been added before use) to the centrifuge tube, shake and mix well, and transfer the suspension to a new 1.5 mL centrifuge tube.
- 1.6. Fix the centrifuge tube on the magnetic device and let it stand for 1 minute, then discard the solution.
- 1.7. Add 1 mL of washing buffer 1 (please check whether ethanol has been added before use) to the centrifuge tube, vortex for 5 seconds, and then put it on a constant temperature mixer at 25°C and 1600 rpm for 2 minutes.
- 1.8. Fix the centrifuge tube on the magnetic device and let it stand for 1 minute, then discard the solution.
- 1.9. Add 1 mL of washing buffer 2 (please check whether ethanol has been added before use) to the centrifuge tube, vortex for 5 seconds, and then put it on a constant temperature mixer at 25°C and 1600 rpm for 2 minutes.
- 1.10. Fix the centrifuge tube on the magnetic device and let it stand for 1 minute, then discard the solution.
- 1.11. Repeat steps 1.9-1.10.
- 1.12. After centrifuging the centrifuge tube for a short time, fix it on the magnetic device again and use a pipettor to remove the solution at the bottom of the tube. Open the cover and place it at room temperature for 5-10 minutes to fully volatilize the ethanol.