Huwel Blood RNA Extraction Kit

HL-GRX-100: 100 Exts

PI/HLGRX-00

Intended use

Huwel Blood RNA Extraction Kits are intended for molecular biology applications such as PCR, RT-PCR, and sequencing. These kits are not intended for the diagnosis, prevention, or treatment of a disease.

Background Information

The Huwel Blood RNA Extraction Kit is a fast, economical and easy method of genomic RNAisolation from blood or tissue. The buffer system provided in the kit allows efficient removal of RBC and lysis of WBC followed byselective binding of nucleic acids to the spin column. These kits produce high quality and yield of Blood RNA.

Kit components

Component	Quantity	Storage
10X RBC Lysis Buffer	50 mL	RT
Spin Column	100	RT
Collection tube	200	RT
Huwel Lysis Buffer	40 mL	RT
Huwel Wash Buffer 1	50 mL	RT
Huwel Wash Buffer 2	2 x 50 mL	RT
Huwel Elution Buffer	20 mL	RT
Lyophilized Poly A	500 µg	-15°C to -25°C
Proteinase K	100 mg	-15°C to -25°C
Kit Protocol	1	NA

Note: Huwel Wash Buffer 1 and Huwel Wash Buffer 2 are provided with Ethanol. No need to add additional Ethanol. PBS is not provided.

Add 10 µl Beta Mercapto Ethanol (β-ME) or 20 µl of 2 M dithiothreitol (DTT) per 1 ml of HuwelLysis Buffer before use.

Prepare 1X RBC Lysis Buffer from 10X RBC Lysis Buffer.

Storage

- Huwel Blood RNA Extraction Kits are stable at room temperature (15–25°C) until the expiration date mentioned on the kit
- **Store the Lyophilized Poly A at 4°C and Lyophilized Proteinase K at -15°C to -25°C upon receipt of the kit**
- Reconstitute the Lyophilized Poly A in 500 µL of Huwel Elution Buffer. Prepare 50 µL aliquots in sterile vials and store at -15°C to -25°C; stable for 12 months

Huwel Blood RNA Extraction Kit

- Reconstitute the Lyophilized Proteinase K in 5 mL of Huwel Elution Buffer. Prepare small aliquots according to the user requirement and store at -15°C to -25°C; stable for 12 months

Equipment, reagents, and materials required but not supplied:

- Micro centrifuge capable of >13,000 x g at room temperature
- 1.5 mL sterile microfuge tubes
- Incubator or dry bath for incubation at 72°C
- 1000 µL,200 µL and 10 µL Pipettes
- 1000 µL and 200 µL and 10 µL Pipette tips
- Disposable powder free gloves
- Vortexer
- Refrigerated centrifuge
- 15mL conical tubes
- Laminar Airflow
- PPE(Lab coat, Gloves, Goggles)
- Beta MercaptoEthanol(β-ME))
- of 2 M dithiothreitol (DTT**)**

Huwel Blood RNA Extraction Kit

Procedure

RBC lysis from whole blood:

- Mix 1 volume of whole blood with 5 volumes of 1x RBC Lysis Buffer.
- Incubate for 10–15 min on ice. Mix by vortexing briefly 2 times during incubation.
- Centrifuge at 400 x g for 10 min at 4°C, and completely remove and discard supernatant.
- Add 2 volumes of 1x RBC Lysis Buffer to the cell pellet. Resuspend cells by vortexing briefly.
- Centrifuge at 400 x g for 10 min at 4°C, and completely remove and discard supernatant.(If required, repeat the step until the clear and white pellets are obtained.)
- Add 400 µl of HuwelLysis buffer to the clear cell pellet. Mix thoroughly by vortex or pipetting to remove any clumps and proceed to Nucleic Acid extraction.

RNA extraction:

1. To 400 µL of cell lysate in Huwel Lysis Buffer, add 5 µL of **Poly A** and 50 µL of **Proteinase K** in a microfuge tube.
2. Mix well and incubate for 10 min at 72°C
3. Add 100 µL of **Huwel Lysis Buffer**, vortex the tube for 5 sec and briefly spin to collect the sample droplets inside the cap
4. Load the entire sample lysate (555 µL) into Huwel spin column-collection tube assembly
5. Centrifuge at 8,000 x g for 1 min. Discard the flowthrough.
6. Change the collection tube, add 500 µL of **Huwel Wash Buffer 1**
7. Centrifuge 8,000 x g for 1 min. Discard the flowthrough
8. Add 450 µL of **Huwel Wash Buffer 2**
9. Centrifuge 8,000 x g for 1 min and discard the flowthrough
10. Add 450 µL of **Huwel Wash Buffer 2**
11. Centrifuge 8,000 x g for 1 min. Discard the flowthrough

Huwel Blood RNA Extraction Kit

12. Change the collection tube (microfuge tube not provided), give empty spin by centrifuging at 13,000 x g for 1 min
13. Place the spin column into a fresh microfuge tube
14. Add 50/100 µL of **Huwel Elution Buffer** into the spin column
15. Incubate the spin column at RT for 5 mins
16. Centrifuge 8,000 x g for 1 min
17. Store the eluent at -20°C

Huwel Blood RNA Extraction Kit

Troubleshooting guide

Problem	Possible cause	Recommendation
Low nucleic acid quality or yield	Buffers stored at sub-optimal conditions	Store the kit at room temperature (15–25°C). Close reagent bottles tightly to avoid contamination and maintain stability. Store the reconstituted Poly A and Proteinase K at -15°C to -25°C.
	Incomplete mixing of sample and reagents	Mix the sample tube well after adding each reagent.
Poor elution of Nucleic acids	Drift in pH of water	If an alternate source of water or buffer is used for elution, make sure that the pH matches with the Elution buffer provided in the kit.
Low RNA yield	RNase contamination	Work in RNase-free environment. Use fresh samples or store the samples at -80°C until they can be processed. Use eluted RNA immediately for downstream applications or store at -80°C.

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