VetExtract (Column)

User Manual

Cat. No. E02-01-1117

Column based extraction for viral nucleic acid from veterinary and environmental samples

Includes main components for 50 extractions

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HAI KANG LIFE CORPORATION LIMITED

PLEASE READ THROUGH THE ENTIRE PROTOCOL BEFORE STARTING.

1. KIT COMPONENTS

- 1 x 30 ml Buffer V-A
- 1 x 7 ml Buffer V-B
- 1 x 7 ml Buffer AP2
- 1 x 24 ml Buffer W1A
- 1 x 24 ml Buffer W2
- 1 x 4 ml Buffer TE (DNase/RNase free)
- 50 x Nucleic acid prep tubes
- 50 x 2 ml microfuge tubes
- 50 x 1.5 ml microfuge tubes

Storage Conditions

Store the kit at room temperature.

Precautions

- Avoid contact with skin or clothing. Conduct extraction within chemical fume hood. Avoid breathing harmful vapour.
- The following guidelines should be observed when working with RNA to prevent the introduction of RNases:
  - Always wear disposable gloves.
  - Use sterile, disposable plasticware and pipets reserved for RNA work.
  - Non-disposable glassware should be baked at 180°C for 4 hours.

Preparation of Reagents

- Buffers W1A and W2 require the addition of ethanol with amount indicated on the bottle label. Store this buffer at room temperature afterwards.
- To prevent the degradation of viral RNA, please use RNase free pipets and 1.5 ml microcentrifuge tubes, or use after treated with DEPC water. During purification, the extracted RNA should be added with 1 unit/µl RNasin.
- Precautions:
  - Please wear your protective equipment to prevent any infections
  - All wastes should be disposed in a waste blanket which contains sterilizer
  - To prevent the presence of fault positive, please use DNA-free, RNA-free equipment and apparatus
  - Buffer V-A, Buffer AP2 and Buffer W1A contains irritation substances, please wear gloves, goggles and prevent the substances directly contact with your skins, eyes and clothes, or inhaled. If contact with the substances, please wash with water or saline and consult with your doctor.

2. PROCEDURES

This protocol is for preparation of viral nucleic acid from 250 µl body fluid. For other sample volumes, Buffer V-A, Buffer V-B, and Buffer AP2 should be used proportionally to sample volume used.

1. Collect 250 µl into a 1.5-ml microfuge tube.
2. Add 500 µl Buffer V-A, vortex mix well.
3. Incubate for 5 minutes at room temperature.
4. Add 125 µl Buffer AP2 and 125 µl Buffer V-B. Mix vigorously.
5. Centrifuge at 12,000 x *g* for 5 min.
6. Place a kit-provided Nucleic acid prep tube onto a 2-ml microfuge tube.
7. Transfer 850 µl supernatant from Step 5 to the Nucleic acid prep tube.
8. Centrifuge at 5,000 x *g* for 1 min.
9. Discard flow-through in the 2-ml microfuge tube. Place Nucleic acid prep tube onto the 2-ml microfuge tube.
10. Add 700 µl Buffer W1A into Nucleic acid prep tube. Centrifuge at 5,000 x *g* for 1 min.
11. Discard flow-through in the 2-ml microfuge tube. Place Nucleic acid prep tube onto the 2-ml microfuge tube.
12. Add 700 µl Buffer W2 into Nucleic acid prep tube. Centrifuge at 12,000 x *g* for 1 min.
13. Repeat Steps 11 and 12 once more.
14. Place the Nucleic acid prep tube onto a fresh, kit-provided 1.5-ml microfuge tube. Centrifuge at 12,000 x *g* for 1 min.
15. Place the Nucleic acid prep tube onto a fresh, RNase-free 1.5-ml microfuge tube (not provide). Add 60 µl Buffer TE to the center of the column membrane. Incubate at room temperature for 1 to 5 min.
16. Centrifuge at 12,000 x *g* for 1 min to elute the nucleic acid.

Notes:

- For solid sample, such as tissues, feces, etc, homogenization will allow better isolation of RNA. Centrifuge homogenized samples for 10 minutes (12,000 x g) at 2 – 8°C to obtain clearer supernatant for extraction procedure.
- Swab sample can be placed into 800 µl RNase-free saline. Vortex the sample tube soaked with the swab for at least 15 seconds. Transfer as much swab-suspended saline as possible to a new microfuge tube. Use 250 µl for extraction.

3. TECHNICAL ASSISTANCE

Our technical staff will provide technical assistance you may need in using this kit. Simply call +**(852) 2111 2123** during our office hours:

Monday – Friday: 9:00 a.m. to 5:30 p.m.
Saturday: 9:00 a.m. to 1:00 p.m.

A recorded message (in English, Cantonese or Putonghua) may be left at other times. Alternatively, you are welcomed to contact our technical staff by fax or email.

Fax: +(852) 2111 9762
Email: technical@haikanglife.com

4. WARRANTIES AND LIABILITIES

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