VDS
Extraction Plus
Cat. No. E02-01-1116

Extraction reagent for viral RNA from
veterinary and environmental samples

Includes main components for 50 RNA extractions

Rev. 0
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HAI KANG LIFE CORPORATION LIMITED

PLEASE READ THROUGH THE ENTIRE PROTOCOL BEFORE STARTING.

1. KIT COMPONENTS

- 50 x 0.9 ml Lysis Buffer (containing guanidine thiocyanate)
  ☒*Harmful: guanidine thiocyanate is harmful by inhalation, in contact with skin and if swallowed. Contact with acid liberates highly toxic gas. Protect Lysis buffer from excessive heat and light.*
- 1 x 45 ml Wash Buffer 1 (containing guanidine thiocyanate)
  ☒*Harmful: guanidine thiocyanate is harmful by inhalation, in contact with skin and if swallowed. Contact with acid liberates highly toxic gas. Protect Wash buffer from excessive heat and light.*
- 1 x 55 ml Wash Buffer 2
- 1 x 30 ml Wash Buffer 3
- 6 x 0.5 ml Silica Suspension
- 5 x 1 ml Elution Buffer

Storage Conditions
Store all components at 2-15ºC. Freezing may affect extraction efficiency of the kit. Do not store silica suspension in close proximity to magnetic source. Expiry dates shown on the box indicate the date beyond which reagents should no longer be used.

Additional Reagents Required (all molecular grade)
The following materials are required but not supplied:
- We recommend using aerosol resistant tips for fluids containing nucleic acids to prevent cross-contamination of samples.
- RNase-free 1.5 ml or 0.5 ml microfuge tubes
- Vortex
- Table top centrifuge (capacity of 10,000 x g) suitable for 1.5 ml microfuge tubes
- Magnetic rack for 1.5 ml microfuge tubes (eg. Promega MagneSphere® Technology Magnetic Separation Stand (Cat No. Z5342) or Nuclisens Mini Mag extraction instrument).

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 180ºC for 4 hours.

2. PREPARATION OF REAGENTS

Nucleic acid extraction
- Pre-warm a Lysis Buffer vial and Wash Buffer 1 vial for about 30 min at 37ºC before starting the release procedure to make sure that any crystals have dissolved. Allow these buffers to cool to room temperature.
- Bring the Silica and Elution buffer to room temperature before use.
- The kit has been validated and optimized using the Promega MagneSphere® Technology Magnetic Separation Stand (Cat No. Z5342).

Re-use of reagents: If fewer than 10 samples are analysed at one time, the remainder of the isolation reagents may be re-used provided that the opened reagents have been stored at 4ºC for no longer than two months.

3. PROCEDURES
Remarks:
- *Wear gloves throughout the procedure to protect your RNA samples from nucleases.*
- *All procedures should be carried out at room temperature unless otherwise stated.*

1. Spin Lysis Buffer tubes for 30 s at 10,000 x g.
2. Add 100-150 µl sample to a Lysis Buffer tube and invert mix 2-3 times.
3. Vortex the Silica Suspension and immediately add 50 µl Silica Suspension to each Lysis Buffer tube from Step 2.
4. Vortex to mix.
5. Leave the tubes for 10 min at room temperature (no further mixing is required).
6. Centrifuge all tubes for 2 min at 5,000 x g.
7. Place tubes in magnetic rack and allow silica to form pellet.
8. Carefully remove the supernatant (do not disturb the pellets by leaving the tubes on the magnetic rack while aspirating) and add 400 µl Wash Buffer 1 to each tube.
9. Remove tubes from rack and gently invert several times until pellet is fully resuspended.
10. Repeat wash procedures step (7) to (9).
    - Once with 400 µl Wash Buffer 1;
    - Twice with 500 µl Wash Buffer 2;
    - Once with 500 µl Wash Buffer 3.

    Caution: Do not pause while the samples are in Wash Buffer 3. Prolonged exposure to Wash Buffer 3 may result in lower yields of nucleic acid.
11. After the final wash step, carefully remove any residual Wash Buffer 3 with a 100 µl pipet.
12. Add 50 µl elution buffer to each microfuge tube.
13. Remove tubes from rack and gently tap tubes until pellet is fully resuspended.
14. Leave the resuspended silica for 5 min at 60ºC to elute the nucleic acid.
    Note: This step can be carried out on a thermoshaker, if available, otherwise waterbath/heatblock is acceptable.
15. Place tubes in magnetic rack and allow silica to form pellet.
16. Transfer extracted nucleic acid supernatants to a fresh microfuge tube (ensuring no silica is transferred). Use for amplification within 1 hour or store at -80ºC for up to 3 months.

4. 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

5. WARRANTIES AND LIABILITIES