



Instructions for Use

SpinStar™

Viral Nucleic Acid Kit Ultra

SpinStar™

Viral Nucleic Acid Ultra

For simultaneous extraction of viral nucleic acids
(DNA and RNA) from human serum and plasma,
VTM and stool



Product No.: 813803



5 extractions / 100 extractions



Store Proteinase K and Carrier RNA at -20°C;
All other components at room temperature (15 - 30°C)



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1. Intended Use

The SpinStar™ Viral Nucleic Acid Kit Ultra is intended for simultaneous purification of viral DNA and RNA from fresh or frozen human serum and plasma, respiratory specimens in VTM and stool; based on proven silica membrane technology in spin column format. The purified viral DNA and/or RNA is ready to use for downstream analysis, e.g. real-time PCR.

2. Kit Components

Catalog no.	813803
SpinStar™ Ultra columns	100
Collection tubes (2ml)	200
Lysis buffer, SSVL	1 x 24 ml
Wash buffer 1, SSW1*	1 x 28 ml
Wash buffer 2, SSW2*	1 x 18 ml
Carrier RNA (lyophilized)*	2 x 1 mg
Proteinase K	3 x 1.7 ml
Elution buffer, SSE	1 x 20 ml
User Manual	1

**Please refer Section 13. Preparation of Reagents and Buffers for dilution.*

3. Storage

- The SpinStar™ Viral Nucleic Acid Kit Ultra, including the Proteinase K and carrier RNA, is shipped at room temperature (15 - 30°C).
- Upon receipt of the kit, store all components, except Proteinase K and carrier RNA, at room temperature (15 - 30°C). Remove Proteinase K and carrier RNA from the kit box and store them at -20°C. Avoid multiple freezethawing or keeping the Proteinase K / carrier RNA stock solution at room temperature for prolonged periods of time.
- Dissolve carrier RNA in Elution Buffer SSE. Store reconstituted carrier RNA in aliquots and ensure it will only be thawed once. Dissolve carrier RNA should be added to Lysis Buffer SSVL as described on page 8. SSVL-carrier

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RNA solution should be prepared fresh, and unused portion is stable at 2 - 8°C for up to one week.

- Kit components are guaranteed to be stable for 18 months from the date of manufacture.
- Buffer SSVL and SSW1 may exhibit salt precipitation due to cold temperature. Warm the buffer at 55 - 65°C with occasional mixing until all precipitates are completely dissolved.

4. Quality Control

Each lot of SpinStar™ Viral Nucleic Acid Kit Ultra is tested against pre-determined specifications to ensure consistent product quality.

5. Product Use Limitations

The SpinStar™ Viral Nucleic Acid Kit Ultra is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease. All due care and attention should be exercised in the handling of the products.

6. Product Warranty

AstronDX Technologies guarantees the correct function of the SpinStar™ Viral Nucleic Acid Kit Ultra for applications as described in the manual. The user must determine the suitability of the product for the particular intended use. Should the product fail to perform satisfactorily in the described applications, please contact AstronDX Technologies Technical Support (**16. Technical Support**) for troubleshooting.

AstronDX Technologies reserves the right to change, alter, or modify any product to enhance its performance and design.

7. Product Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs).

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries.

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Buffers SSVL and SSW1 contain guanidine salts, which can form highly reactive-compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to components of the SpinStar™ Viral Nucleic Acid Kit Ultra:

Buffer SSVL

Contains guanidine isothiocyanate: harmful. Risk and safety phrases:

*R20/21/22-32-52-53, S13-26-36-37-46

Buffer SSW1

Contains guanidine isothiocyanate: harmful, irritant. Risk and safety phrases:

*R20/21/22-32-52-53, S13-26-36-37-46

Proteinase K

Contains proteinase K: Sensitizer, irritant. Risk and safety phrases:

*R36/37/38-42/43, S22-23-24-26-36/37

*R-phrase(s):

Contains guanidine isothiocyanate: harmful. Risk and safety phrases:

R20/21/22 Harmful by inhalation, in contact with skin and if swallowed

R32 Contact with acids liberates very toxic gas

R36/37/38 Irritating to eyes, respiratory system and skin

R42/43 May cause sensitization by inhalation and skin contact

R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment

*S-phrase(s):

S13 Keep away from food, drink and animal feeding stuffs

S22 Do not breathe dust

S23 Do not breathe vapor

S24 Avoid contact with skin

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S36-37 Wear suitable protective clothing and gloves.

S46 If swallowed, seek medical advice immediately and show container or label

S61 Avoid release to the environment. Refer to special instructions/Safety data sheets

24-hour emergency information

Emergency medical information can be obtained 24 hours a day in English and Malay Language from:

National Poison Centre, Malaysia

Tel: +604-657 0099

For other countries, please visit the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) for a link to the nearest Poison Centre:

<https://www.eapcct.org/index.php?page=links>

8. Introduction

The SpinStar™ Viral Nucleic Acid Kit Ultra uses proven technology of silica-based membrane for simultaneous purification of viral DNA and RNA. The procedure is suitable for use with human plasma and serum, VTM and stool. Samples can either be fresh or frozen, provided that they have not been frozen and thawed more than once. Viral nucleic acids are eluted in Buffer SSE, ready for use in amplification reactions or storage at -20°C. Purified nucleic acids are free of proteins, nucleases, and other impurities.

9. Principle and Procedure

The SpinStar™ Viral Nucleic Acid Kit Ultra procedure comprises 4 steps (lyse, bind, wash, elute) and is carried out using SpinStar™ Ultra columns in a standard micro-centrifuge. The procedure is designed to ensure that there is no sample-to-sample crosscontamination and allows safe handling of potentially infectious samples. The simple SpinStar™ Viral Nucleic Acid Kit Ultra procedure, which is highly suited for simultaneous processing of multiple samples, yields pure nucleic acid in approx. 1 hour. The SpinStar™ Viral Nucleic Acid Kit Ultra can be used for isolation of viral RNA and DNA from a broad range of RNA and DNA viruses. However, performance cannot be guaranteed for every virus species and must be validated by the user.

10. Specimen Storage and Handling

After collection and centrifugation, serum and plasma (from EDTA blood) can be stored at 2 - 8°C for up to 6 hours. For long-term storage, we recommend freezing specimens in aliquots at -20°C or -80°C.

Frozen plasma or serum specimens must not be thawed more than once. Multiple freeze-thawing leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral DNA/RNA. In addition,

cryoprecipitates formed during freeze-thawing might clog the SpinStar™ Ultra column membrane. If cryoprecipitates are visible, they can be pelleted by centrifugation at 6800 x *g* for 3 minutes. The cleared supernatant should be aspirated without disturbing the pellet, and processed immediately.

11. Material and Devices required but Not Provided

- 1.5 ml microcentrifuge tubes (e.g. Eppendorf Safe-Lock Tubes™, cat. no. 0030 120.086) for elution and heat lysis
- 2.0 ml microcentrifuge tubes (for preparation of stool suspension)
- Microcentrifuge ($\geq 17,000 \times g$)
- Pipettes, adjustable (range: 10 μ l, 100 μ l, 200 μ l, 1000 μ l)
- Pipette tips (with aerosol barriers)
- Disposable gloves
- Heating block for lysis of samples at 65°C
- Vortex mixer
- Absolute Ethanol (96 - 100%)
- Measuring cylinder (100ml)
- For sample <200 μ l: sterile 0.9% w/v NaCl solution (for volume fill-up)
- PBS (pH7.4) for preparation of stool suspension

12. Important Notes and Precautions

- Use of this product is limited to personnel specially trained in the techniques of nucleic acids extraction.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.

- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) specimen preparation, (ii) reaction set-up and (iii) amplification/detection activities. Workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not use components of the kit that have passed their expiration date.
- Discard extraction waste according to your local safety regulations.

13. Preparation of Reagents and Buffers

Prepare the following before using every new SpinStar™ Viral Nucleic Acid Kit Ultra for the first time:

1. Carrier RNA - Add 1 ml SSE and mix well. Use 15 µl carrier RNA per sample by adding it into 200 µl SSVL and invert gently 10x to mix. Store carrier RNA solution at -20°C in aliquots.
2. Wash Buffers - Add 28 ml absolute ethanol to SSW1 and 42 ml absolute ethanol to SSW2. Shake to mix.

14. Protocol

Things to do before starting

- Pre-set heating block to 65°C.
- Completely thaw and equilibrate specimens to room temperature. Briefly vortex and quick-spin samples before use.
- Label all tubes and SpinStar™ Ultra Columns (placed in Collection Tubes).

- Freshly prepare SSVL-carrier RNA mix (prepare 10% excess).

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- The centrifugation steps are referenced to the Heraeus Pico 21 microcentrifuge (Thermo Fisher).
- All steps are carried out at room temperature (15 - 30°C).

14.1 Protocol for Preparation of Stool Suspension

1. Add approx. 20 mg stool into 2.0 ml PBS (1X). Suspend by vortex mixing.
2. Centrifuge at 8,000 x g (9,000 rpm) for 1 min.
3. Use 200 µl supernatant for extraction according to Section 14.2.

14.1.1 Preparation of PBS, 1X (1000 ml)

1. Mix the following to 800 ml double-distilled water (ddH₂O) and dissolve completely with a magnetic stirrer:

8.0 g NaCl
0.2 g KCl
1.44 g Na₂HPO₄
0.24 g KH₂PO₄
2. Adjust pH to 7.4 with 1M HCl
3. Top up to 1000 ml with ddH₂O and autoclave.

14.2 Protocol for Human Serum, Plasma and VTM

1. Pipette 50 µl Proteinase K into the bottom of a 1.5 ml microcentrifuge tube.
2. Add 200 µl sample to the microcentrifuge tube and mix.
3. Add 215 µl Lysis Buffer SSVL-carrier RNA mix to the sample. Pulse-vortex for 15s.
4. Incubate at 65°C for 10 min.
5. Briefly centrifuge the 1.5 ml microcentrifuge tube.

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6. Add 280 µl absolute ethanol to the sample and pulse-vortex for 15 s.
7. Transfer all mixture from Step 6 (approx. 745 µl) to a SpinStar™ Ultra column without wetting the rim. Close the cap and centrifuge at 6200 x g (8000 rpm) for 1 min. Place the SpinStar™ Ultra column in a clean Collection tube and discard the tube containing the filtrate.
8. Carefully open the SpinStar™ Ultra column and add 500 µl Wash Buffer 1, SSW1 without wetting the rim. Close the cap and centrifuge at 6200 x g (8000 rpm) for 1 min. Place the SpinStar™ Ultra column in a clean Collection tube, and discard the tube containing the filtrate.
9. Carefully open the SpinStar™ Ultra column and add 500 µl Wash Buffer 2, SSW2 without wetting the rim. Close the cap and centrifuge at 6200 x g (8000 rpm) for 1 min.
10. Discard filtrate and reuse Collection tube. Carefully open the SpinStar™ Ultra column and add 500 µl absolute ethanol without wetting the rim. Close the cap and centrifuge at 6200 x g (8000 rpm) for 1 min.
11. Discard filtrate and reuse Collection tube. Centrifuge at 17,000 x g (13,300rpm) for 10 min.
12. Transfer the SpinStar™ Ultra column to a clean 1.5 ml microcentrifuge tube and discard the Collection tube containing trace ethanol.
13. Carefully open the SpinStar™ Ultra column and add 30 - 60 µl Elution Buffer SSE on the center of the membrane. Incubate at room temperature (15 - 30°C) for 5 min, then centrifuge at 6200 x g (8000 rpm) for 1 min. Proceed to downstream (e.g. real-time PCR) reaction setup or store eluate at -20°C if not used immediately.

15. Troubleshooting

Issues	Possible Causes	Comments & Suggestions
Low amounts of DNA/RNA	Insufficient lysis	- Proteinase K was subjected to multiple freeze-thaw or elevated temperature for a prolonged time. ➤ Repeat by using fresh Proteinase K on new specimens
	Insufficient binding of DNA / RNA to the membrane	- Carrier RNA degraded / insufficient carrier RNA or carrier RNA not added to Buffer SSL ➤ Use carrier RNA reconstituted in Buffer SSE stored at -20°C in aliquots ➤ Use freshly prepared Buffer SSVL-carrier RNA mix
	DNA / RNA degraded	- DNA degraded by RNases in specimens / RNases contaminated buffers ➤ Extract specimens promptly following collection or removal from storage ➤ Repeat extraction with new Buffers and specimens - Specimens freeze-thawed more than once / very old specimens ➤ Use fresh starting material or frozen specimens at -20°C / -80°C
	Low viral copy / nucleic acid concentration in specimens	- High elution volume used ➤ Elute with lower volume of Buffer SSE - Incomplete elution ➤ Use buffer SSE preheated to 70°C ➤ Elute in 2x 50µl SSE ➤ Use nuclease-free water to elute viral RNA
PCR inhibition observed in downstream application (e.g. in real-time PCR)	Ethanol carryover in eluate	Ensure that the ethanol removal step is being carried out at 17,000 x g for 10 min (Protocol step no. 11)

16. Technical Support

For customer support, please contact our Technical Support:

e-mail: techsupport@astrondx.com

phone: +603 7931 6760

17. Appendix

Explanation of Symbols

Symbol	Explanation
	<i>In vitro</i> diagnostic medical device
	Product Number
	Batch Code
	Manufacturer
	Contains sufficient for “n” tests/rxns
	Temperature limitation
	Version
	Use-By Date

18. Ordering Information

Products	Packing (extractions)	Order No.
SpinStar™ Viral Nucleic Acid Kit Ultra	100	813803
SpinStar™ Viral Nucleic Acid Kit 1.0	100	811803
SpinStar™ Pathogen Nucleic Acid Kit 1.0	100	814803
SpinStar™ Total DNA Kit 2.0	100	821803
SpinStar™ VNAplus Mix	100	812803

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