Instructions for Use

SpinStar™
Total DNA Kit 2.0

SpinStar™
Total DNA Kit 2.0

For extraction of total DNA
from human whole blood, plasma, serum,
sputum, VTM, stool, cultured cells and tissues

REF Product No.: 821803

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™ Total DNA Kit 2.0 is intended for purification of total DNA (e.g. genomic, mitochondrial and of bacterial, viral and parasitic origin) from up to 400 µl human whole blood, serum, plasma, respiratory specimen in VTM and up to 200 µl sputum (pretreated), stool suspension, cultured cells (up to 5 x 10^6) or various tissues (e.g. kidney, heart, lungs, brain, muscles, liver, spleen, etc.); based on proven silica membrane technology in spin column format. The purified DNA is ready to use for downstream analysis, e.g. real-time PCR.

2. Kit Components

Catalog no.	821803
SpinStar™ columns	100
Collection tubes (2ml)	300
Tissue Lysis buffer, **TL**	30 ml
Lysis Enhancer	2 ml
Genomic DNA Binding Buffer, **CB**	64 ml
Wash buffer 1, (concentrate)*	30 ml
Wash buffer 2, (concentrate)*	34 ml
Elution buffer	20 ml
Proteinase K	2 x 1.05 ml
User Manual	1

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

3. Storage

- The SpinStar™ Total DNA Kit 2.0, including the Proteinase K, is shipped at room temperature (15 - 30°C).

- Upon receipt of the kit, store all components, except Proteinase K, at room temperature (15 - 30°C). Remove Proteinase K from the kit box and store them at -20°C. Avoid multiple freezethawing or keeping the Proteinase K stock solution at room temperature for prolonged periods of time.

- Kit components are guaranteed to be stable for 18 months from the date of manufacture.

4. Quality Control

Each lot of SpinStar™ Total DNA Kit 2.0 is tested against pre-determined specifications to ensure consistent product quality.

5. Product Use Limitations

The SpinStar™ Total DNA Kit 2.0 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™ Total DNA Kit 2.0 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 date 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.

Buffers CB 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™ Total DNA Kit 2.0:

Buffer CB
Contains guanidine hydrochloride: harmful, irritant. Risk and safety phrases:
*R22-36/38, S13-26-36-46

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™ Total DNA Kit 2.0 uses proven technology of silica-based membrane for purification of total DNA from human whole blood, serum, plasma, sputum, VTM, stool, cultured cells and tissues. DNA is eluted in Elution Buffer, 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™ Total DNA Kit 2.0 procedure comprises 4 steps (lyse, bind, wash, elute) and is carried out using SpinStar™ columns in a standard microcentrifuge. The procedure is designed to ensure that there is no sample-to-sample cross-contamination and allows safe handling of potentially infectious samples. The simple SpinStar™ Total DNA Kit 2.0 procedure, which is highly suited for simultaneous processing of multiple samples, yields pure nucleic acid in approx. 1 hour (depending on sample type). The SpinStar™ Total DNA Kit 2.0 can be used for purification of total DNA from up to 400 µl whole blood, serum, plasma, VTM and up to 200 µl sputum (pretreated), stool suspension in PBS, cultured cells (up to 5 x 10^6) or various tissues (10 - 20 mg).

10. Specimen Storage and Handling

After collection, whole blood (in EDTA or citrate tubes) can be stored at 2 - 8°C for up to 24 hours. For long-term storage, we recommend freezing specimens in aliquots at -20°C or -80°C.

Multiple freeze-thawing leads to denaturation and precipitation of proteins, resulting in reduced yields of DNA. In addition, cryoprecipitates formed during freeze-thawing might clog the SpinStar™ 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 (≥ 17,000 x *g)*

- Pipettes, adjustable (range: 10 µl, 100 µl, 200 µl, 1000 µl)
- Pipette tips (with aerosol barriers)
- Disposable gloves
- Heating shaker block for lysis of samples at 65°C / 100°C (sputum)
- Vortex mixer
- Absolute Ethanol (96 - 100%)
- Measuring cylinder (100ml)
- For sample <200 µl: sterile 0.9% w/v NaCl solution (for volume fill-up)
- PBS (pH7.4) for preparation of stool suspension
- RNase A (DNase-free), 20 mg / ml

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 diferrent 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™ Total DNA Kit 2.0 for the first time:

For 822801 (5 preps):

1. Add **1.5 ml** absolute Ethanol to **Wash Buffer 1** and **5.6 ml** absolute Ethanol to **Wash Buffer 2**. Cap the bottles tightly, shake to mix and store at room temperature.

For 822803 (100 preps):

1. Add **30 ml** absolute Ethanol to **Wash Buffer 1** and **80 ml** absolute Ethanol to **Wash Buffer 2**. Cap the bottles tightly, shake to mix and store at room temperature.

14. Protocol

Things to do before starting

- Pre-set heating block to 65°C and another heating block at 100°C (for MTB sputum samples).
- Pre-heat Elution Buffer at 65°C
- Completely thaw and equilibrate specimens to room temperature. Invert and quick-spin samples before use.
- Label all tubes and SpinStar™ columns (placed in Collection Tubes).

Notes

- The centrifugation steps are referenced to the Heraeus Pico 21 microcentrifuge (Thermo Fisher).

- All centrifugation 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. For viral DNA extraction, centrifuge at 8000 x *g* (9,000 rpm) for 1 min.

3. For bacterial extraction, centrifuge at 500 x *g* (2,300 rpm) for 1 min.

3. Use 200 µl supernatant from No. 2 or No. 3 for extraction according to Section 14.3.

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 Na_2HPO_4
   0.24 g KH_2PO_4

2. Adjust pH to 7.4 with 1M HCl

3. Top up to 1000 ml with ddH_2O and autoclave.

4. Store at room temperature.

14.2 Protocol for Pretreatment of Sputum (for MTB extraction)

1. Transfer up to 10 ml sputum in a 50 ml centrifuge tube.

2. Add an equal volume of NALC-NaOH solution (refer to 14.2.1 for preparation).

3. Incubate at room temperature for 20 min under rotation / gentle shaking.

4. Add sterile Phosphate buffer (refer to 14.2.1) to a final volume of 45 ml and add 2 - 3 drops 5% Tween 80 solution. Invert several times to mix.

5. Centrifuge at 3500 x *g* (6000 rpm) for 20 - 30 min.

6. Carefully decant all supernatant.

7. Resuspend pellet in 0.5 - 2.0 ml of Phosphate buffer.

8. Use 200 µl suspension for DNA extraction (Section 14.3)

14.2.1 Preparation of NALC-NaOH and Phosphate buffer

i) **NALC-NaOH (100ml)***

50 ml, 4% NaOH (sterile)

50 ml, 2.9% Na-Citrate-Dihydrate (sterile)

50 ml, N-Acetyl-L-Cystein (NALC)

NOTE

Use NALC-NaOH solution within 24 hours after preparation

NaOH/Na-Citrate mixture may be used for 3 months. Prepare 0.5 g NALC aliquots and simply add one of them to 100ml NaOH/Citrate mixture directly before starting the decontamination procedure.

ii) **Phosphate buffer (1000ml)***

4.74 g Na_2HPO_4 (anhydrous)

4.54 g KH_2PO_4 (anhydrous)

Add 1000 ml distilled water and autoclave

*Alternatively, ready-to-use NALC-NaOH solution and Phosphate buffer can be purchased from BD (BBL™ MycoPrep™ Specimen Digestion / Decontamination Kit, catalogue no. BD240862).

14.3 Protocol for Human Whole Blood, Serum, Plasma, VTM

1. Add 200 µl Buffer CB to 200 µl sample in a 1.5 ml microcentrifuge tube. Mix

thoroughly by pulse-vortex.
If using >200 µl blood sample, increase the volumes of Buffer BB, Proteinase K and Wash Buffer proportionally. Using >400 µl blood sample is not recommended.

2. Add 20 µl Proteinase K and mix immediately.

3. Incubate at 65°C for 10 min.
Incubate for a further 10 min at 100°C for **MTB sputum samples** *and cool to room temperature before proceeding to Step 4.*

4. Briefly centrifuge the 1.5 ml microcentrifuge tube.
Optional: Removal of RNA: If RNA-free DNA is required, add 20 µl RNase A. Mix and incubate at 37°C for 10 min.

5. Add 200 µl absolute ethanol to the sample and mix immediately by pulse-vortex.

6. Transfer all mixture from Step 5 (approx. 620 µl) to a SpinStar™ column without wetting the rim. Close the cap and centrifuge at 6200 x *g* (8000 rpm) for 1 min. Place the SpinStar™ column in a clean Collection tube and discard the tube containing the filtrate.
Note: If processing Tissue samples, repeat this step to load all remaining lysate.

7. Carefully open the SpinStar™ column and add 500 µl Wash Buffer 1, without wetting the rim. Close the cap and centrifuge at 6200 x *g* (8000 rpm) for 1 min. Place the SpinStar™ column in a clean Collection tube, and discard the tube containing the filtrate.

8. Carefully open the SpinStar™ column and add 500 µl Wash Buffer 2, without wetting the rim. Close the cap and centrifuge at 6200 x *g* (8000 rpm) for 1 min.

9. Discard filtrate and reuse Collection tube.

10. Centrifuge at 17,000 x *g* (13,300 rpm) for 10 min.

11. Transfer the SpinStar™ column to a clean 1.5 ml microcentrifuge tube and discard the Collection tube containing trace Wash Buffer 2.

13. Carefully open the SpinStar™ column and add 60 µl pre-heated Elution Buffer to the center of the membrane. Incubate at room temperature (15 - 25°C) for 2 min, and then centrifuge at 9600 x *g* (10,000 rpm) for 1 min.

14.4 Protocol for Cultured Cells

1. Pellet appropriate amount of cells (up to 5 x 10^6) in a clean 1.5 ml microcentrifuge tube by centrifugation at 800 x *g* for 5 min at 4°C. Decant supernatant.
   If the starting specimen is frozen cell pellet, thaw the cells completely on ice.

2. Add 20 µl Proteinase K and 2 µl Lysis Enhancer to the sample and mix thoroughly by pulse-vortex. Briefly centrifuge the tubes.

3. Add 200 µl Buffer CB and mix thoroughly by pulse-vortex.

4. **Proceed to Step 3 (Section 14.3).**

14.5 Protocol for Tissues

1. Cut 10 - 20 mg tissue sample into small pieces with a clean scalpel.
   Tissue sample can be ground into fine powder with a pestle and mortar (in liquid nitrogen) or using a glass tube homogenizer (in buffer TL), for a more efficient lysis.

2. Add 250 µl Buffer TL and 20 µl Proteinase K to the sample and mix thoroughly by pulse-vortex.

3. Add 12 µl Lysis Enhancer and mix immediately.

4. Incubate at 65°C for 1 - 3 hours (extent to overnight incubation if tissue mixture does not appear clear) in a shaking heater. Vortex sample frequently during incubation if a shaking heater is not available.

5. Add 560 µl buffer CB and mix thoroughly by pulse-vortex until a homogenous solution is obntained. Briefly centrifuge the tubes.

6. **Proceed to Step 3 (Section 14.3).**

15. Troubleshooting

Issues	Possible Causes	Comments & Suggestions
Low amounts of DNA	Tissue sample not thoroughly homogenized	• Ensure tissues are completely homogenized
	Blood sample is not stored properly	• Store blood sample in small aliquots to avoid multiple freeze-thaw cycles
	Insufficient lysis	• Ensure Buffer BB and blood sample are mixed by pulse-vortex before adding Proteinase K • Ensure Proteinese K is mixed homogeneously with Buffer BB-blood sample mixture • Proteinese 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 to the membrane	• Ensure that ethanol is added prior to loading lysate to spin column
	DNA degraded	• Specimens freeze-thawed more than once / very old specimens ➢ Use fresh starting material or new aliquots of frozen specimens
	Elution volume not optimum	• High elution volume used ➢ Elute with lower volume of Elution Buffer • Incomplete elution ➢ Use Elution Buffer preheated to 65°C ➢ Elute in 2x 50µl Elution Buffer
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. 10)

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
	*In vitro* 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™ Total DNA Kit 2.0	100	821803
SpinStar™ Pathogen Nucleic Acid Kit 1.0	100	814803
SpinStar™ Viral Nucleic Acid Kit 1.0	100	811803
SpinStar™ Viral Nucleic Acid Kit Ultra	100	813803
SpinStar™ VNAplus Mix	100	812803

NOTES

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