

Instruction for Use

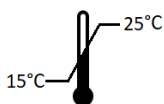
STAR BEADS Universal DNA/RNA Extraction Kit



SBK270,1x16 - SBK270,1x96 -
SBK271,1X96PFI - SBK273,2X32PFI



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May 2024/Rev 06

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About us

Cyanagen is a biotech company located in Bologna, dedicated to research, development, and production of reagents for life science since 2003 and one of the leading companies in reagents for Western blotting and ELISA. Since 2020 the company entered the IVD market with nucleic acids extraction kits based on proprietary magnetic beads.

The main product lines are focused on chemiluminescent and fluorescent substrates and nucleic acids extraction kits for biological analysis, genomics, proteomics and molecular diagnostics. Most of Cyanagen products are based on Cyanagen's internationally patented technologies and achieve outstanding performance in terms of sensitivity and stability.

Customer satisfaction and product top quality are of paramount importance to us.

Cyanagen Srl has a certified Quality System

ISO 9001:2015 QUALITY CERTIFIED

ISO 13485:2016 QUALITY CERTIFIED

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1. General information

1.1. Description

STAR BEADS Universal DNA/RNA Extraction Kit provides a fast and efficient purification method to isolate high-quality nucleic acids from a wide variety of specimens for reliable downstream application.

The easy procedure of STAR BEADS Universal DNA/RNA Extraction Kit can be used on automatic magnetic separators, with the dedicated extraction protocol. The procedure's actual time depends on the instrument's configuration and the magnetic separation system used. The amount of total nucleic acids recovery depends on the type of sample and on pre-analytical sample handling.

1.2. Intended use

STAR BEADS Universal DNA/RNA Extraction Kit is designed, developed and tested for isolation of nucleic acids from a wide variety of specimens such as biological fluids, cultured cells, formalin-fixed-paraffin-embedded (FFPE) samples, frozen and fresh tissues, small-size organisms i.e. insects and food. The kit is "Research Use Only" (RUO) and should not be used in diagnostics procedures. The product has not been tested for drug development and it is unsuitable for administration to humans or animals.

The product is intended for use by professionals such as technicians, doctors and biologists trained in molecular biology techniques only. It is designed to be used with any downstream application that employs enzymatic amplification or other enzymatic modifications of DNA/RNA followed by signal detection or amplification.

1.3. Principle

The procedure is based on the reversible adsorption of nucleic acids to STAR BEADS Magnetic beads in appropriate buffers, while impurities are effectively removed during the washing steps.

Sample lysis and binding of nucleic acids to STAR BEADS Magnetic Beads are performed in STAR BEADS Lysis/Binding Buffer with the addition of STAR BEADS Proteinase K.





After magnetic separation, the magnetic beads are washed with two special washing reagents (STAR BEADS Washing Buffer 1 and STAR BEADS Washing Buffer 2) to remove contaminants and salts. Finally, purified nucleic acids are eluted with STAR BEADS Elution Buffer which causes the nucleic acid to detach from the magnetic beads. The resulting high-quality total nucleic acids are then ready for use in downstream applications such as RT-PCR, PCR, sequencing, or any other type of enzymatic reaction, or they can be frozen.

2. Components and other required materials

2.1. Kit content

The Kit is available in both bottle format as well as prefilled plates.





Bottle format: REF SBK270,1X16 (sample size), SBK270,1X96. Use in combination with Allsheng Auto-Pure 96, Procomcure Phoenix-Pure 96, MOLGEN PurePrep 96, Thermo Fisher Scientific KingFisher™ Flex, Allsheng Auto-Pure32 A, Allsheng Auto-Pure Mini, Procomcure Phoenix-Pure 32, MOLGEN PurePrep 32, BIOER GenePure Pro NPA-32P, MGI SP-NE32, BIGFISH BFEX-32, 3D Med ANDiS 350.

Components	REF	GHS	Kit size (1x16 preps) SBK270,1x16 (sample size)	Kit size (96 preps) SBK270,1x96
STAR BEADS Lysis/Binding Buffer*	SBPLBB249		15 mL	85 mL
STAR BEADS Washing Buffer 1*	SBWC242		10 mL	55 mL
STAR BEADS Washing Buffer 2	SBWC243		10 mL	55 mL
STAR BEADS Elution Buffer	SBEB228	None	3 mL	15 mL
STAR BEADS Proteinase K	SBK263,0750		0.75 mL	3 x 0.75 mL

* Contains chaotropic salt. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents that contain bleach. See Material Safety Data Sheet for safety information.

NB. Please note that components from different batches cannot be used interchangeably.





Prefilled All Inclusive format: REF SBK271,1X96PFI. Use in combination with Allsheng Auto-Pure 96, Procomcure Phoenix-Pure 96, MOLGEN PurePrep 96, Thermo Fisher Scientific KingFisher™ Flex.

Components	REF	GHS
STAR BEADS Sample Plate (1) – Prefilled with STAR BEADS Lysis – Binding Buffer SBPLBB249*	SBSP252,1X96PFI	
STAR BEADS Washing 1 Plate (1) – Prefilled with STAR BEADS Washing Buffer 1 SBWC242*	SBWP192,1X96PF	
STAR BEADS Washing 2 Plate (1) – Prefilled with STAR BEADS Washing Buffer 2 SBWC243	SBWP193,1X96PF	
STAR BEADS Elution Plate (1)– Prefilled with STAR BEADS Elution Buffer SBEB228	SBEP230,1X96PFI	None
STAR BEADS Tip Comb Plate (1)	SBTP196,1X96PF	None
STAR BEADS Proteinase K (3x 0,75 mL)	SBK263,0750	

* Contains chaotropic salt. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents that contain bleach. See Material Safety Data Sheet for safety information.

NB. Please note that components from different batches cannot be used interchangeably.

Prefilled All Inclusive format: REF SBK273,2X32PFI. Use in combination with Allsheng Auto-Pure32 A, Allsheng Auto-Pure Mini, Procomcure Phoenix-Pure 32, MOLGEN PurePrep 32, BIOER GenePure Pro NPA-32P, MGI SP-NE32, BIGFISH BFEX-32 and 3D Med ANDiS 350.

Components	REF	GHS
STAR BEADS Extraction Plate (4) * Prefilled with: <ul style="list-style-type: none"> - STAR BEADS Lysis - Binding Buffer SBPLBB249 - STAR BEADS Washing Buffer 1 SBWC242 - STAR BEADS Washing Buffer 2 SBWC243 - STAR BEADS Elution Buffer SBEB228 	SBK251,1X16PFI	  
STAR BEADS Proteinase K (2x 0,75mL)	SBK263,0750	

* Contains chaotropic salt. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents that contain bleach. See Material Safety Data Sheet for safety information.

NB. Please note that components from different batches cannot be used interchangeably.

The kit is to be used in combination with dedicated equipment and plastic consumables. See in section 2.3.3

2.2. Shipping and storage

The kit and all its components are shipped and must be stored at room temperature (RT) (+15 to +25°C). Do not use the Product after the expiry date indicated on the label. The pre-filled plates should be stored with the part closed by the aluminum foil facing upwards.

2.3. User supplied equipment and materials

2.3.1. Equipment required for manual extraction

- Micropipettes suitable for pipetting 10-20 µL, 150 µL, 300 µL, 500 µL
- Vortex
- Magnetic separation plate or magnet for separating magnetic beads
- DNase / RNase-free tubes or plates- Disposable tips without DNase / RNase (filter tips recommended)
- Very low temperature freezer for the storage of samples stored at -80 ° C
- Biological hood suitable for working with potentially infectious samples. Follow local guidelines for working with potentially infectious material, particularly if derived from a human or animal sample.

2.3.2. Equipment needed for automated extraction

The supplied kit is compatible with automatic magnetic separators. The equipment required may vary depending on the instrument used.

Required equipment includes:

- Personal Protective Equipment (PPE): Please follow local guidelines for working with potentially infectious material, particularly if derived from a human or animal sample.
- Freezer for the storage of STAR BEADS Carrier once reconstituted with STAR BEADS Carrier Buffer at -20 ° C.
- Ultra-low temperature freezer for the storage of samples stored at -80 ° C.
- Biological Safety Cabinet suitable for handling potentially infectious samples. Follow local guidelines for working with potentially infectious material, particularly if derived from a human or animal sample.

2.3.3 Plastic consumables needed for automated extraction (to be ordered separately)

STAR BEADS Universal DNA/RNA Extraction Kit, SBK273,2X32PFI is to be used in combination with:

- **STAR BEADS Rod's Tips REF. SBK198,1X2 (a bag with two rod's tips)** for Allsheng Auto-Pure32 A, Allesheng Auto-Pure Mini, Procomcure Phoenix-Pure 32, MOLGEN PurePrep 32 (to be ordered separately)
- **STAR BEADS Rod's Tips B REF. SBK240,1X2 (a bag with two rod's tips)** for BIOER GenePure Pro NPA-32P, MGI SP-NE32, BIGFISH BFEF-32 (to be ordered separately)
- **STAR BEADS Rod's Tips C REF. SBK303,1X2 (a bag with two rod's tips)** for 3D Med ANDiS 350 (to be ordered separately)

2.3.4 Reagents needed for sample preparation

- **STAR BEADS Lysis Buffer** (code SBPLB254) for samples requiring a digestion step (to be ordered separately).
- **STAR BEADS Carrier** (code SBC268,001 or SBC268,0005) and **STAR BEADS Carrier Buffer** (code SBCB269,0012), to increase the yield of microbial DNA/RNA extraction (to be ordered separately).
- **Phosphate Buffered Saline (PBS)** depending on the sample type.

2.3.5 Equipment needed for sample preparation

Depending on the sample type, a dedicated equipment may be required:

- Heating block
- Homogenization tools
- Centrifuge

3. Sample preparation

3.1 Before starting

For samples requiring a digestion step, **order STAR BEADS Lysis Buffer (code SBPLB254)**.

STAR BEADS Lysis Buffer may form salt precipitates upon storage below 15-25°C. If any precipitate forms, incubate the buffer bottle at 40 °C until all the precipitates are re-dissolved.

To increase the yield of pMicrobial DNA/RNA from samples such as biological fluids, feces or tissues, order **STAR BEADS Carrier** code SBC268,001 or SBC268,0005 and **STAR BEADS Carrier Buffer** code SBCB269,0012.

To reconstitute the STAR BEADS Carrier (1mg/mL), centrifuge the lyophilized contents of the vial, carefully open the vial cap avoiding any dispersion of the powder and dissolve with:

- 1 mL of STAR BEADS Carrier Buffer in STAR BEADS Carrier REF SBC268,001
- 0,5 mL of STAR BEADS Carrier Buffer in STAR BEADS Carrier REF SBC268,0005

Mix well until completely dissolved, using a vortex. For a complete dissolution of STAR BEADS Carrier, it is recommended to mix well with a vortex and not making up and down with the pipette. If STAR BEADS Carrier is not completely dissolved, leave the vial at room temperature and mix every 5 minutes with vortex until completely dissolved.

After the reconstitution, it is recommended to store STAR BEADS Carrier in aliquots at **-20 °C for up to 6 months**.

3.2 Cultured cells

Collect cells according to the appropriate culture conditions. Resuspend cell pellet (1×10^5 to 1×10^6 cells) in 200 μ L of PBS and proceed following the Protocol in sections 4-5-6 (manual/automated procedure).

3.3 Biological Fluids (serum, plasma, saliva, urine, whole blood, cerebrospinal fluid)

Use up to 200 μL aliquot and proceed with the Protocol in sections 4-5-6 (manual/automated procedure). If the sample volume is less than 200 μL , adding an appropriate volume of PBS is recommended. For increased yield of microbial nucleic acids, use **STAR BEADS Carrier** (see section 3.1 for its preparation).

3.4 Tissues

In a clean empty tube, disrupt up to 25 mg of fresh or frozen tissues and small-size organisms such as insects depending on the sample type (e.g., pestle, drill, mortar or liquid nitrogen). Grind or mechanically homogenize the sample to reduce the lysis time and increase the yield of nucleic acids purified.

Add up to 300 μL of **STAR BEADS Lysis Buffer** to the lysate sample.

Add 20 μL of **STAR BEADS Proteinase K**. Vortex the sample for 10 seconds and briefly centrifuge the tube. Incubate at 60 °C for 1h.

Note: lysis time may vary depending on the type of tissue processed. To ensure efficient lysis wait until the tissue is completely dissolved and vortex occasionally during incubation.

Centrifuge at 12'000 RPM for 1 min at room temperature to precipitate possible residue to the bottom of the microcentrifuge tube, and collect 200 μL of the supernatant.

Continue extraction following the Protocol in sections 4-5-6 (manual/automated procedure). For increasing the yield of microbial nucleic acids, use **STAR BEADS Carrier** (see section 3.1 for its preparation).

3.5 Feces and fecal swabs

Please refer to applicable guidelines for collection, handling and storage of samples and other sample preparation requirements or continue following the procedure below:

- in a clean empty 2 mL microtube add 500 μ L of **STAR BEADS Lysis Buffer REF. SBPLB254,0030** and 250 μ L of liquid feces/250 mg of solid feces.
- Add **40 μ L** of **STAR BEADS Proteinase K**. (to be ordered separately, cod. SBK263). Vortex the sample for 10 seconds and briefly centrifuge the tube.
- Incubate at **95°C for 5 minutes**, vortex and wait 1 minute at RT.
- Incubate at **56°C for 10 minutes**, vortex and wait 1 minute at RT.
- Centrifuge at 12'000 RPM for 1 min at room temperature to precipitate possible residue to the bottom of the micro centrifuge tube, and collect 300 μ L of the supernatant.

Continue extraction following the Protocol in sections 4-5-6 (manual/automated procedure). For increasing the yield of microbial nucleic acids, use **STAR BEADS Carrier** (see section 3.1 for its preparation).

3.6 FFPE tissues

This protocol is suitable for genomic DNA extraction from at least 3 sections ($\geq 4 \mu\text{m}$) of FFPE tissue per sample. The yield, purity and length of DNA isolated from FFPE samples strongly depend on the type of tissue, age of specimen, fixation and inclusion procedure as well as reagents used. Tissue cross-linking due to formalin fixation and paraffin embedding, often make it difficult to extract long, amplifiable DNA fragments. Thus, consider targeting genomic regions of 200 nucleotides or less for downstream applications.

- Dewax FFPE sample following laboratory practices.
Once paraffin is removed from the specimen, collect at least 3 sections ($\geq 4 \mu\text{m}$) of tissue per sample with 300 μ L of **STAR BEADS Lysis Buffer** in a new 1.5 mL microcentrifuge tube (not provided)

Note: if the specimen is on a glass microscope slide, once deparaffinized, dispense 150 μ L of **STAR BEADS Lysis Buffer** on the slice and then use another 150 μ L to include any remains and collect all the tissue from the slide

- Add 20 μ L of **STAR BEADS Proteinase K**
- Vortex the sample for 10 seconds and briefly centrifuge the tube
- Incubate at 60 °C for 1h. After incubation with Proteinase K, visible debris may be present, but they will not affect DNA extraction. Prolonged digestion at 60°C doesn't seem to increase the final yield
- Briefly centrifuge the tube to collect possible drops
- For cross-linking removal, incubate the sample at 90°C for at least 1h. Incubation time depends on several parameters like sample type as well as fixation and embedding procedures. Long-hour incubation could improve amplifiable DNA recovery.
- Vortex the sample for 10 seconds
- Cool down the sample at room temperature
- Centrifuge at 12'000 RPM for 5 min at room temperature to precipitate possible residue to the bottom of the microcentrifuge tube, and collect up to 300 μ L of the supernatant.
- Continue extraction following the Protocol in sections 4-5-6 (manual/automated procedure).

3.7 Swabs

For dry swab, place the dry swab in 400 μ L of **STAR BEADS Lysis Buffer**. Add 20 μ L of **STAR BEADS Proteinase K** and vortex the sample for 10 seconds. Incubate at 56°C for 20 minutes.

Vortex the sample for 10 seconds and collect up to 300 μ L .

Continue extraction following the Protocol in sections 4-5-6 (manual/automated procedure). For increasing the yield of microbial nucleic acids, use **STAR BEADS Carrier** (see section 3.1 for its preparation).

For swabs in Universal Transport Media or other preservation solution, incubate at RT the swab for 30 minutes with gentle shaking to release sample

material. Use a 200 μ L aliquote to proceed with the Protocol in sections 4-5-6 (manual/automated procedure). If the sample volume is less than 200 μ L, adding an appropriate volume of PBS is recommended. For increasing the yield of microbial nucleic acids, use **STAR BEADS Carrier** (see section 3.1 for its preparation).

4. Protocol for the isolation of nucleic acids (manual procedure with bottle format REF. SBK270,1X16 – SBK270,1X96)

4.1 Lyse the sample and bind nucleic acid

In a clean empty tube, add **up to 300 μ L of sample** obtained as previously described in section 3 to **820 μ L of STAR BEADS Lysis/Binding Buffer**.

In case it has not been previously added during sample preparation described in section 3, dispense **20 μ L of STAR BEADS Proteinase K**.

(Optional) For increasing the yield of microbial nucleic acids, add **4 μ L STAR BEADS Carrier** (see in section 3.1 for its preparation).

Mix well by vortexing and inverting the tube up and down several times for 10 min at room temperature. Briefly centrifuge the sample and after 5 minutes of separation on the magnetic support remove the supernatant. Note: optimization may be required for incubation time and incubation temperature, depending on the sample type.

The user must validate the STAR BEADS Universal DNA/RNA Extraction Kit in combination with the consumables used and the downstream application. Appropriate controls (e.g. internal controls, extraction controls, positive / negative controls) must be used. For the internal negative control, use 200 μ L of nuclease-free water instead of the sample.

4.2 Wash magnetic beads

Add **500 μ L STAR BEADS Washing Buffer 1** and mix well by vortexing and inverting the tube up and down several times. Briefly centrifuge the sample

and after 2-3 minutes of separation on the magnetic support remove the supernatant.

4.3 Wash magnetic beads

Add **500 µL STAR BEADS Washing Buffer 2** and mix well by vortexing and inverting the tube up and down several times. Briefly centrifuge the sample and after 2-3 minutes of separation on the magnetic support remove the supernatant.

4.4 Wash magnetic beads (optional)

Add **500 µL ethanol (96-100%)** and mix well by vortexing and inverting the tube up and down several times. Briefly centrifuge the sample and after 2-3 minutes of separation on the magnetic support remove the supernatant.

4.5 Dry magnetic beads

Incubate at room temperature for 5-10 min until the magnetic beads are dried, avoiding excessive drying.

4.6 Elute highly pure nucleic acids

Add **100 µL STAR BEADS Elution Buffer** and mix well by vortexing and inverting the tube up and down several times for 5 minutes. It is essential to cover the magnetic beads completely with elution buffer during this step.

4.7 Collect nucleic acids

Separate 2-3 min on the magnetic support and transfer supernatant containing the eluted nucleic acids into a new DNase/RNase free plate/tube.

5. Protocol for the isolation of nucleic acids (automated procedure, with bottle format REF. SBK270,1X16 - SBK270,1X96)

5.1. Automated extraction with STAR BEADS Universal DNA/RNA Extraction kit, bottle format REF. SBK70,1X16, SBK270,1X96 in combination with Allsheng AutoPure 96, Procomcure Phoenix-Pure96, MOLGEN PurePrep 96

- Set up the plates using standard 96 deep well plates and 96 Tip combs compatible with the relative instrument. Prepare the Sample, Washing 1, Washing 2 and Elution plates according to the following table:

Plate	Component	Reagent volume per well
Sample Plate	STAR BEADS Lysis – Binding Buffer REF. SBPLBB249	820 µL
Washing 1 Plate	STAR BEADS Washing Buffer 1 REF. SBWC242	500 µL
Washing 2 Plate	STAR BEADS Washing Buffer 2 REF. SBWC243	500 µL
Elution Plate	STAR BEADS Elution Buffer REF. SBEB228	100 µL

- Add **up to 300 µL of sample** according to sample preparation in section 3 to the relevant wells of the Sample Plate, starting from the well in position A1.
- Depending on the sample preparation described in section 3, dispense **20 µL of STAR BEADS Proteinase K**, in case it has not been

previously added to the relevant wells of the Sample Plate, starting from the well in position A1.

- (Optional) For increasing the yield of microbial nucleic acids, add **4 µL STAR BEADS Carrier** (see in section 3.1 for its preparation) to the relevant wells of the Sample Plate, starting from the well in position A1.
- Add the appropriate Extraction Controls* to the relevant wells of the Sample Plate.
- Turn on the extractor.
- Make sure you have downloaded the correct extraction protocol ** to the instrument.
- Load the plates into the instrument in the correct position as indicated in the table. Place the A1 well of each plate in the corner marked as A1 in each station of the turntable:

Plate	Position
Tip Comb Plate	1
Sample Plate	2
Washing 1 Plate	3
Washing 2 Plate	4
Elution Plate	8

- Press “Run”.
- After the extraction session is complete, remove the Elution plate from position 8 of the instrument and proceed with downstream applications.

* Appropriate controls (e.g. internal controls, extraction controls, positive / negative controls) should be used, according to the downstream assay. For internal negative control, use 200 µL of nuclease-free water instead of sample.

** Download the script for the relative extraction system from documents section on <https://www.cyanagen.com/products/star-beads-universal-dna-rna-extraction-kit-bottle-prefilled-plate-format/>
For related technical support email to technical.support@cyanagen.com

5.2. Automated extraction with STAR BEADS Universal DNA/RNA Extraction kit, bottle format REF. SBK270,1X16, SBK270,1X96 in combination with Allsheng Auto-Pure32 A, Allsheng Auto-Pure Mini, Procomcure Phoenix-Pure 32, MOLGEN PurePrep 32, BIOER GenePure Pro NPA-32P, MGI SP-NE32, BIGFISH BFEF-32, 3D Med ANDiS 350

- Use standard 96 deep well plates and Rod's tips compatible with the relative instrument. Prepare up to two Extraction plates outside the instrument according to the following table:

Column	Component	Volume per well
1-7	STAR BEADS Lysis – Binding Buffer REF. SBPLBB249	820 µL
2-8	STAR BEADS Washing Buffer 1 REF. SBWC242	500 µL
3-9	STAR BEADS Washing Buffer 2 REF. SBWC243	500 µL
6-12	STAR BEADS Elution Buffer REF. SBEB228	100 µL

- Add **up to 300 µL of sample** according to sample preparation in section 3 to the relevant wells in column 1/7 of the Extraction Plate.
- Depending on the sample preparation described in section 3, dispense **20 µL of STAR BEADS Proteinase K** in case it has not been previously added in the relevant wells of the Extraction Plate.
- (Optional) For increasing the yield of microbial nucleic acids, add **4 µL STAR BEADS Carrier** (see in section 3.1 for its preparation) to the relevant wells in column 1/7 of the Extraction Plate.
- Add the appropriate Extraction Controls * to the appropriate wells of the Extraction Plate.
- Turn on the extractor.
- Make sure you have downloaded the correct protocol ** on the instrument.

- Insert a new Rod's Tip into the instrument (Be sure that the Rod's Tip is compatible with the relative instrument, see section 2.3.3, and to replace the Rod's Tip with a new one to avoid any contamination). The number of Rod's tips depends on the number of Extraction Plate (2 Rod's Tip for each Extraction Plate).
- Place the Extraction Plate in the instrument, in the same position where the Rod's tip were previously inserted and with the labels attached to the plates facing the operator.
- Press "Run".
- After the extraction session is over, remove the Extraction plate from the instrument, recover the purified nucleic acid from columns 6 and 12 and proceed with the downstream applications.

* Appropriate controls (e.g. internal controls, extraction controls, positive / negative controls) should be used, according to the downstream assay. For internal negative control, use 200 μ L of nuclease-free water instead of sample.

** Download the script for the relative extraction system from documents section on <https://www.cyanagen.com/products/star-beads-universal-dna-rna-extraction-kit-bottle-prefilled-plate-format/>

For related technical support email to technical.support@cyanagen.com

5.3. Automated extraction with STAR BEADS Universal DNA/RNA Extraction kit, bottle format REF. SBK270,1X16, SBK270,1X96 in combination with Thermo Fisher Scientific KingFisher™ Flex.

- Set up the plates using standard 96 deep well plates and 96 Tip combs compatible with the relative instrument. Prepare the Sample, Washing 1, Washing 2 and Elution plates according to the following table:

Plate	Component	Reagent volume per well
Sample Plate	STAR BEADS Lysis – Binding Buffer REF. SBPLBB249	820 μ L
Washing 1 Plate	STAR BEADS Washing Buffer 1 REF. SBWC242	500 μ L
Washing 2 Plate	STAR BEADS Washing Buffer 2 REF. SBWC243	500 μ L
Elution Plate	STAR BEADS Elution Buffer REF. SBEB228	100 μ L

- Add **up to 300 μ L of sample** according to sample preparation in section 3 to the relevant wells of the Sample Plate, starting from the well in position A1.
- Depending on the sample preparation described in section 3, dispense **20 μ L of STAR BEADS Proteinase K**, in case it has not been previously added to the relevant wells of the Sample Plate, starting from the well in position A1.
- (Optional) For increasing the yield of microbial nucleic acids, add **4 μ L STAR BEADS Carrier** (see in section 3.1 for its preparation) to the relevant wells of the Sample Plate, starting from the well in position A1.
- Add the appropriate Extraction Controls* to the relevant wells of the Sample Plate.
- Turn on the extractor.
- Make sure you have downloaded the correct extraction protocol ** to the instrument.
- Start the run and load the plates into the indicated position when prompted by the instrument.
- After the extraction session is complete, remove the Elution plate from the instrument and proceed with downstream applications.

* Appropriate controls (e.g. internal controls, extraction controls, positive / negative controls) should be used, according to the downstream assay. For internal negative control, use 200 μ L of nuclease-free water instead of sample.

** Download the script for the relative extraction system from documents section on <https://www.cyanagen.com/products/star-beads-universal-dna-rna-extraction-kit-bottle-prefilled-plate-format/>

For related technical support email to technical.support@cyanagen.com

6. Protocol for the isolation of nucleic acids with prefilled format (REF. SBK271,1X96PFI - SBK273,2X32PFI)

6.1. Before using the pre-filled plates

Before each use of the pre-filled plates check the integrity of the plate.

6.2. Automated extraction with STAR BEADS Universal DNA/RNA Extraction kit, Prefilled Plates format REF. SBK271,1X96PFI in combination with Allsheng Autopure 96, Procomcure Phoenix-Pure96, MOLGEN PurePrep 96.

- Centrifuge the plate for a few seconds or shake downward with a sharp blow by hand to prevent the reagents from adhering to the well walls.
- Remove the aluminum foil from the STAR BEADS Sample Plate. Orient the plate so that the label faces the operator.
- Add **up to 300 μ L of sample** according to sample preparation described in section 3 starting from the well in position A1.
- Depending on the sample preparation described in section 3, dispense **20 μ L of STAR BEADS Proteinase K** in case it has not been

previously added in the relevant wells of the STAR BEADS Sample Plate starting from the well in position A1.

- (Optional) For increasing the yield of microbial nucleic acids, add **4 µL STAR BEADS Carrier** (see in section 3.1 for its preparation) to the relevant wells of the STAR BEADS Sample Plate, starting from the well in position A1.
- Add the appropriate Extraction Controls* to the relevant wells of the STAR BEADS Sample Plate starting from the well in position A1.
- Turn on the extractor.
- Make sure you have downloaded the correct extraction protocol ** to the instrument
- Remove the aluminum foil from the STAR BEADS Washing 1 Plate , STAR BEADS Washing 2 Plate, STAR BEADS Elution Plate. Load all the plates into the instrument in the correct position as indicated in the table. Place the A1 well of each plate in the corner marked as A1 in each station of the turntable:

Plate	Position
STAR BEADS Tip Comb Plate	1
STAR BEADS Sample Plate	2
STAR BEADS Washing 1 Plate	3
STAR BEADS Washing 2 Plate	4
STAR BEADS Elution Plate	8

- Press "Run".
- After the extraction session is complete, remove the **STAR BEADS Elution plate from position 8** of the instrument and proceed with downstream applications.

* Appropriate controls (e.g. internal controls, extraction controls, positive / negative controls) should be used, according to the downstream assay. For internal negative control, use 200 µL of nuclease-free water instead of sample.

** Download the script for the relative extraction system from documents section on <https://www.cyanagen.com/products/star-beads-universal-dna->

[rna-extraction-kit-bottle-prefilled-plate-format/](#)

For related technical support email to technical.support@cyanagen.com

6.3. Automated extraction with STAR BEADS Universal DNA/RNA Extraction kit, Prefilled Plates REF. SBK273,2X32PFI in combination with Allsheng Auto-Pure32 A, Allsheng Auto-Pure Mini, Procomcure Phoenix-Pure 32, MOLGEN PurePrep 32, BIOER GenePure Pro NPA-32P, MGI SP-NE32, BIGFISH BFEK-32, 3D Med ANDiS 350

- Centrifuge the plate for a few seconds or shake downward with a sharp blow by hand to prevent the reagents from adhering to the well walls.
- Remove the aluminum foil from the STAR BEADS Extraction Plate. Orient the plate so that the label faces the operator, with the wells of column 1 containing magnetic beads on the left side.
- Add **up to 300 μ L of sample** according to sample preparation described in section 3 to the relevant wells in column 1/7 of the STAR BEADS Extraction Plate.
- Depending on the sample preparation described in section 3, dispense **20 μ L of STAR BEADS Proteinase K** in case it has not been previously added in the corresponding wells in column 1/7 of the STAR BEADS Extraction Plate.
- (Optional) For increasing the yield of microbial nucleic acids, add **4 μ L STAR BEADS Carrier** (see in section 3.1 for its preparation) to the corresponding wells in column 1/7 of the STAR BEADS Extraction Plate.
- Add the appropriate Extraction Controls * to the appropriate wells of the STAR BEADS Extraction Plate.
- Turn on the extractor.
- Make sure you have downloaded the correct protocol ** on the instrument.

- Insert a new Rod's Tip into the instrument (Be sure that the Rod's Tip is compatible with the relative instrument, see section 2.3.3, and to replace the Rod's Tip with a new one to avoid any contamination). The number of Rod's tips depends on the number of Extraction Plate (2 Rod's Tip for each Extraction Plate).
- Place the STAR BEADS Extraction Plate in the instrument, in the same position where the Rod's tip were previously inserted and with the labels attached to the plates facing the operator.
- Press "Run".
- After the extraction session is over, remove the STAR BEADS Extraction Plate from the instrument, recover the **purified nucleic acid from columns 6 and 12** and proceed with the downstream applications.

* Appropriate controls (e.g. internal controls, extraction controls, positive / negative controls) should be used, according to the downstream assay. For internal negative control, use 200 μ L of nuclease-free water instead of sample.

** Download the script for the relative extraction system from documents section on <https://www.cyanagen.com/products/star-beads-universal-dna-rna-extraction-kit-bottle-prefilled-plate-format/>

For related technical support email to technical.support@cyanagen.com

6.4. Automated extraction with STAR BEADS Universal DNA/RNA Extraction kit, Prefilled Plates format REF. SBK271,1X96PFI in combination with Thermo Fisher Scientific KingFisher™ Flex.

- Centrifuge the plate for a few seconds or shake downward with a sharp blow by hand to prevent the reagents from adhering to the well walls.
- Remove the aluminum foil from the STAR BEADS Sample Plate. Orient the plate so that the label faces the operator.
- Add **up to 300 µL of sample** according to sample preparation described in section 3 starting from the well in position A1.
- Depending on the sample preparation described in section 3, dispense **20 µL of STAR BEADS Proteinase K** in case it has not been previously added in the relevant wells of the STAR BEADS Sample Plate starting from the well in position A1.
- (Optional) For increasing the yield of microbial nucleic acids, add **4 µL STAR BEADS Carrier** (see in section 3.1 for its preparation) to the relevant wells of the STAR BEADS Sample Plate, starting from the well in position A1.
- Add the appropriate Extraction Controls* to the relevant wells of the STAR BEADS Sample Plate starting from the well in position A1.
- Turn on the extractor.
- Make sure you have downloaded the correct extraction protocol ** to the instrument
- Remove the aluminum foil from the STAR BEADS Washing 1 Plate , STAR BEADS Washing 2 Plate, STAR BEADS Elution Plate. Start the run and load the plates into the indicated position when prompted by the instrument.
- After the extraction session is complete, remove the **STAR BEADS Elution plate from the instrument** and proceed with downstream applications.

* Appropriate controls (e.g. internal controls, extraction controls, positive / negative controls) should be used, according to the downstream assay. For

internal negative control, use 200 μ L of nuclease-free water instead of sample.

** Download the script for the relative extraction system from documents section on <https://www.cyanagen.com/products/star-beads-universal-dna-rna-extraction-kit-bottle-prefilled-plate-format/>

For related technical support email to technical.support@cyanagen.com

7. Troubleshooting

For Bottle format:

Problem	Possible Cause	Precautions/Remedies
Low or inconsistent yield	Low nucleic acids concentration	Concentrate eluted DNA using speedvac or under biological hood at least for 36 h (to be optimized)
	Insufficient elution buffer volume	Beads pellet must be entirely covered with elution buffer
	Insufficient performance of elution buffer during the elution step	Remove Ethanol from the final washing step entirely before proceeding with elution
	Magnetic beads over-drying	The magnetic beads should be free from any visible liquid ethanol but not completely dried out. Reduce drying time
	Loss of magnetic beads	Increase time for magnetic separation and decrease aspiration speed
Magnetic beads carryover	Magnetic separation time too short	Increase separation time

Low purity of nucleic acids	Insufficient washing procedure	Use only the appropriate separator and plates combination. Ensure that the magnetic beads are re-suspended during the washing. If the agitation is not sufficient to re-suspend entirely, mix repeatedly
	Evaporation of Ethanol from Wash buffer	Close the bottles of the buffer well, avoiding the evaporation of the Ethanol
Poor performance of DNA/RNA in downstream applications	Ethanol carryover	The magnetic beads should be free from any visible liquid ethanol before the elution step
	Beads carryover	Increase the time on the magnetic separator during elution step
	DNA/RNA degradation	Avoid any nuclease contamination
	Too low concentration	From relative wells, remove up to 40 µL of elution buffer
Low reproducibility of DNA/RNA extraction	STAR BEADS Lysis Buffer forms salt precipitates if stored below 20-25 °C	Incubate the buffer bottle at 40 °C until all of the precipitates are re-dissolved
	STAR BEADS Washing Buffer 1 forms salt precipitates if stored below 20-25 °C	Incubate the buffer bottle at 40 °C until all of the precipitates are re-dissolved

For prefilled plates:

Problem	Possible Cause	Precautions/Remedies
Low or inconsistent yield	Low nucleic acids concentration	Concentrate eluted DNA using speedvac or under biological hood at least for 36 h (to be optimized)


	Plates stored at inappropriate temperature	Check the integrity of the plate. Make sure that the plates are stored at room temperature (+ 15-25 °C). If the presence of precipitate is observed in the STAR BEADS Extraction Plate, STAR BEADS Sample Plate, STAR BEADS Washing 1 Plate, incubate at + 40 °C until the precipitate is completely dissolved.
	Plates stored upside down	Check the integrity of the plate. Make sure that the plates are stored in the correct position (the closed side with the aluminum sheet facing up). Centrifuge the plates before removing the aluminum foil to avoid reagent residues sticking to the underside of the aluminum foil.
	Eluate recovered from wrong wells	For the SBK273,2X32PFI format make sure to recover the eluates from columns 6 and 12 from the STAR BEADS Extraction Plate For the SBK271,1X96PFI format be sure to retrieve eluates from the STAR BEADS Elution Plate.
	Error in the positioning of the plates in the extractor	For the SBK273,2X32PFI format, be sure to insert the STAR BEADS Extraction Plate with the label facing the operator (with the wells of column 1 containing magnetic beads on the left side.) For the SBK271,1X96PFI format, make sure to insert the plates in the extractor in the correct position indicated in section 6.2
	Incorrect sample processing	Be sure to add STAR BEADS Proteinase K in the amount indicated.
Poor performance of DNA/RNA in downstream applications	Beads carryover	After completing the extraction procedure, incubate the plate on a magnetic separator for at least 10 minutes
	Too low concentration	From relative wells, remove up to 40 µL of elution buffer

8. Warning and Precautions


- When working with chemicals, always wear protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.). For more information, please refer to the appropriate material safety data sheets (MSDSs) available online at www.cyanagen.com\MSDS\
- Clinical samples and other specimens to be tested should be considered as potentially infectious substances and processed strictly according to laboratory biosafety requirements.
- Components from different batches cannot be used interchangeably. Do not collect reagents from other bottles of the same lot. After use, immediately close all bottles to avoid leakage, changes in buffer concentrations or buffer contamination. After the first opening, keep all the bottles in an upright position.
- Do not use a kit after the expiration date.
- Avoid any nuclease contamination. Always wear gloves and change them often, especially after contact with skin, hair or other potentially nuclease-contaminated surfaces. Use nuclease-free solutions and nuclease-free certified, disposable plastic ware and filter tips. Maintain a separate area for nucleic acids work. Carefully clean all surfaces.
- Do not add bleach or acidic solutions directly to STAR BEADS Lysis-binding Buffer, STAR BEADS Magnetic Beads, STAR BEADS Washing Buffer 1, STAR BEADS Sample Plate, STAR BEADS Washing 1 Plate and STAR BEADS Extraction Plate. They contain guanidine salts, which can form highly reactive compounds when combined with bleach. If the liquid containing these buffers is spilled, clean it with suitable laboratory detergent and water.
- Cyanagen has not tested the liquid waste generated by the STAR BEADS Universal DNA/RNA Extraction Kit procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety


regulations.

- In case of spillage or damage to the bottles, dispose of the components as chemical waste according to local safety regulations.
- Should a user detect the Product's malfunction concerning the stated specifications, download the claim form at <https://www.CYANAGEN.com/cyanacontent/uploads/Pages-content/Support/support-request-form1.pdf>, fill and submit it to CYANAGEN, technical.support@CYANAGEN.com, for internal quality analysis.
- STAR BEADS Universal DNA/RNA Extraction Kit, REF SBK270,1X16 - SBK270,1X96


Name: STAR BEADS Lysis - Binding Buffer – SBPLBB249 – (1X16) 15mL – (1X96) 85mL	
Danger	
Contains:	Guanidine Thiocyanate, PROPAN-2-OL
H225	Highly flammable liquid and vapour.
H314	Causes severe skin burns and eye damage.
H336	May cause drowsiness or dizziness.
EUH032	Contact with acids liberates very toxic gas.
EUH071	Corrosive to the respiratory tract.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P260	Do not breathe dust / fume / gas / mist / vapours / spray.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P303+P361+ P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P310	Immediately call a POISON CENTER / doctor.
P280	Wear protective gloves/ protective clothing / eye protection / face protection.

Name: STAR BEADS Washing Buffer 1 - SBWC242 – (1X16) 10mL – (1X96) 55mL	
Danger	
H225	Highly flammable liquid and vapour.
P403+P235	Store in a well-ventilated place. Keep cool.
P303+P361+ P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P306+P360	IF ON CLOTHING: rinse immediately contaminated clothing and skin with plenty of water before removing clothes.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P241	Use explosion-proof electrical/ventilating/lighting equipment.
P210	Keep away from heat/sparks/open flames/hot surfaces. – No smoking.


Name: STAR BEADS Washing Buffer 2 – SBWC243 – (1X16) 10mL – (1X96) 55mL	
Danger	
H225	Highly flammable liquid and vapour.


P370+P378	In case of fire: Use carbon dioxide, foam, chemical powder for extinction.
P210	Keep away from heat/sparks/open flames/hot surfaces. – No smoking.
P233	Keep container tightly closed.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P303+P361+P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P241	Use explosion-proof electrical/ventilating/lighting equipment.


Name: STAR BEADS Proteinase K - SBK263 – (1X16) 0,75mL – (1x96) 2,25 mL	
Danger	
Contains:	Proteinase K
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P342+P311	If experiencing respiratory symptoms: call a POISON CENTER / doctor.
P304+P340	IF INHALED: remove person to fresh air and keep comfortable for breathing.


- STAR BEADS Universal DNA/RNA Extraction Kit, Prefilled Plates REF SBK271,1X96PFI - SBK273,2X32PFI

Name: STAR BEADS Lysis - Binding Buffer – SBPLBB249 - (1X96) 78,72mL - (2X32) 52,48mL
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Danger	
Contains:	Guanidine Thiocyanate, PROPAN-2-OL
H225	Highly flammable liquid and vapour.
H314	Causes severe skin burns and eye damage.
H336	May cause drowsiness or dizziness.
EUH032	Contact with acids liberates very toxic gas.
EUH071	Corrosive to the respiratory tract.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P260	Do not breathe dust / fume / gas / mist / vapours / spray.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P310	Immediately call a POISON CENTER / doctor.
P280	Wear protective gloves/ protective clothing / eye protection / face protection.

Name: STAR BEADS Washing Buffer 1 - SBWC242 - (1X96) 48mL - (2X32) 32mL	
Danger	
H225	Highly flammable liquid and vapour.
P403+P235	Store in a well-ventilated place. Keep cool.

P303+P361+ P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P306+P360	IF ON CLOTHING: rinse immediately contaminated clothing and skin with plenty of water before removing clothes.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P241	Use explosion-proof electrical/ventilating/lighting equipment.
P210	Keep away from heat/sparks/open flames/hot surfaces. – No smoking.
Name: STAR BEADS Washing Buffer 2 – SBWC243 - (1X96) 48mL - (2X32) 32mL	
Danger	
H225	Highly flammable liquid and vapour.
P370+P378	In case of fire: Use carbon dioxide, foam, chemical powder for extinction.
P210	Keep away from heat/sparks/open flames/hot surfaces. – No smoking.
P233	Keep container tightly closed.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P303+P361+ P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P241	Use explosion-proof electrical/ventilating/lighting equipment.

Name: STAR BEADS Proteinase K - SBK263 - (1X96) 2,25 mL - (2X32) 1,5mL	
Danger	
Contains:	Proteinase K
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P342+P311	If experiencing respiratory symptoms: call a POISON CENTER / doctor.
P304+P340	IF INHALED: remove person to fresh air and keep comfortable for breathing.

Contact and technical support information:
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9. Ordering information

PRODUCT	ORDER - NO	COMPATIBLE EXTRACTORS	UNIT SIZE
STAR BEADS Universal DNA/RNA Extraction Kit	SBK270,1X16	Allsheng Auto-Pure 96, Procomcure Phoenix-Pure 96, MOLGEN PurePrep 96, Thermo Fisher Scientific KingFisher™ Flex, Allsheng Auto-Pure32 A, Allsheng Auto-Pure Mini, Procomcure Phoenix-Pure 32, MOLGEN PurePrep 32, BIOER GenePure Pro NPA-32P, MGI SP-NE32, BIGFISH BFEX-32, 3D MED Andis 350	16 preps
	SBK270,1X96	Allsheng Auto-Pure 96, Procomcure Phoenix-Pure96, MOLGEN PurePrep 96, Thermo Fisher Scientific KingFisher™ Flex	96 preps
	SBK271,1X96PFI	Allsheng Auto-Pure 32 A and Mini, Procomcure Phoenix-Pure 32, MOLGEN PurePrep 32, BIOER GenePure Pro NPA-32P, MGI SP-NE32, BIGFISH BFEX-32, 3D MED Andis 350	96 preps
	SBK273,2X32PFI	Allsheng Auto-Pure 32 A and Mini, Procomcure Phoenix-Pure 32, MOLGEN PurePrep 32, BIOER GenePure Pro NPA-32P, MGI SP-NE32, BIGFISH BFEX-32, 3D MED Andis 350	64 preps

For further information
visit **www.cyanagen.com**
contact **technical.support@cyanagen.com**
For orders: **sales@cyanagen.com**

Warranty Disclaimer at
www.cyanagen.com/warranty-disclaimer/