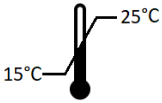
CYANAGEN
Reagents for Molecular Biology

Instruction for Use

STAR BEADS RNA Extraction Kit

REF SBK308,2X32PFI – SBK308,1X10 – SBK308,1X96

Cyanagen srl, Via Andrea Costa 4/2- 40134 Bologna, Italy



Store at +15-25°C

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CYANAGEN
Reagents for Molecular Biology

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 RNA Extraction Kit provides a fast and efficient purification method to isolate high-quality RNA from a wide variety of specimens for reliable downstream applications.
The STAR BEADS Kit is based on magnetic bead technology and it can be used for rapid manual extraction and also for automated extraction on automatic magnetic separators, with the dedicated extraction protocol. The procedure's time depends on the instrument's configuration and the magnetic separation system used. The amount of RNA recovery depends on the type of sample and pre-analytical sample handling.

1.2. Principle

The procedure is based on the reversible adsorption of nucleic acids to STAR BEADS Magnetic beads in appropriate buffers, while impurities are efficiently removed during the washing steps.
Lysis of the sample is performed in STAR BEADS Lysis Buffer 1 1 with the addition of STAR BEADS Reductant. Binding of nucleic acids to STAR BEADS Magnetic Beads is performed in Isopropanol. DNA is digested using a recombinant DNase and RNA is rebound thanks to isopropanol. After magnetic separation, the magnetic beads are washed with two washing reagents (STAR BEADS Washing Buffer 1G and STAR BEADS Washing Buffer 2G) to remove contaminants and salts. Finally, purified RNA is eluted with STAR BEADS Elution Buffer which causes the nucleic acid to detach from the magnetic beads. The resulting high-quality RNA is then ready for use in downstream applications such as RT-PCR, qRT-PCR, sequencing, or any other type of enzymatic reaction, or it can be frozen for later use.

1.3. Intended use

STAR BEADS RNA Extraction Kit is intended for use for the extraction of high-quality total RNA from cultured cells. 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 STAR BEADS RNA Extraction kit is intended to be used at a temperature between + 15°C and 25°C. Use outside of this temperature range may result in suboptimal results.

2. Components, shipping and storage conditions and other required materials

2.1. Kit content

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

Bottle format: REF SBK308,1X10 SBK308,1X96. Use for manual procedure or in combination with Allsheng Auto-Pure 96, Allsheng Auto-Pure Mini, Allsheng Auto-Pure32 A, BIOER GenePure Pro NPA-32P, BIGFISH BFEX 32.

Components	REF	GHS	Kit size (10 preps) SBK308, 1X10 (sample size)	Kit size (96 preps) SBK308, 1X96
STAR BEADS Lysis Buffer 1 *	SBLB187		4 mL	38 mL
STAR BEADS Magnetic Beads	SBB188		0,4 mL	4 mL
STAR BEADS Washing Buffer 1G*	SBWB284		8 mL	70 mL
STAR BEADS Washing Buffer 2G	SBWB285		20 mL	190 mL
STAR BEADS Elution Buffer	SBEB228	None	1,5 mL	12 mL
STAR BEADS Reductant	SBR314		0,1 mL	0,6 mL

STAR BEADS DNase	**SBD315**	**None**	**0,8 mL**	**0,8 mL**
STAR BEADS DNase Dilution Buffer	**SBDDB316**	**None**	**1 mL**	**1 mL**
STAR BEADS DNase Reaction Buffer	**SBDRB317**	**None**	**3,5 mL**	**35 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.

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

Prefilled All Inclusive format: REF SBK308,2X32PFI. Use in combination with Allsheng Auto-Pure Mini, Allsheng Auto-Pure32 A, BIOER GenePure Pro NPA-32P, BIGFISH BFEX 32.

Components	REF	GHS
STAR BEADS RNA Extraction Plate (4) **Prefilled with:** Lines 1 and 7: STAR BEADS Magnetic Beads SBB188 (30 µL/well); Isopropanol (350 µL/well) - Lines 2 and 8: - empty - Lines 3 and 9: STAR BEADS Washing Buffer 1G SBWB284 (600 µL/well) Lines 4 and 10: STAR BEADS Washing Buffer 2G SBWB285 (900 µL/well) Lines 5 and 11- STAR BEADS Washing Buffer 2G SBWB285 (900 µL/well) - Lines 6 and 12: STAR BEADS Elution Buffer SBEB228 (100 µL/well)	**SBK313,1X16PFI**	
STAR BEADS Lysis Buffer 1*, 30 mL	**SBLB187,0030**	
STAR BEADS Reductant	**SBR314, 0,6 mL**	
STAR BEADS DNase	**SBD315**	
STAR BEADS DNase Dilution Buffer	**SBDDB316, 1 mL**	
STAR BEADS DNase Reaction Buffer	**SBDRB317, 20 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.

Note: 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

2.2. Preparation of STAR BEADS DNase working solution

When using the kit for the first time, dilute **STAR BEADS DNase** in **STAR BEADS DNase Dilution Buffer**. Mix by gently pipetting. Do not vortex.
Dispense reconstituted DNase working solution into aliquots and store at -20 °C. The frozen working solution is stable for 6 months. Do not freeze / thaw the aliquots more than three times.

2.3. Shipping and storage

The kit is shipped at room temperature (RT) (+15 to 25°C). All the components can be stored at RT, except for **STAR BEADS Reductant** and **STAR BEADS DNase** that must be stored at -20° C. **STAR BEADS DNase** must be stored at -20°C after reconstitution in **STAR BEADS DNase Dilution Buffer**. Do not use the product after the expiry date indicated on the label.
Do not store the pre-filled plates upside down, but with the side closed with the aluminum sheet facing upwards.

2.4. RNA eluates stability

For short-term storage, it is recommended to store at -20°C. For long-term storage, it is recommended to store at -80°C.

2.5. Required materials to be supplied by the user

- Micropipettes suitable for pipetting 10-20 µL, 150 µL, 300 µL, 500 µL;
- Disposable tips with DNase / RNase (filter tips recommended);
- Refrigerator at 4°C or low/very low temperature freezer at -20/-80°C for the storage of samples;
- Biological hood suitable for working with hazardous, infectious or biologically contaminated materials. Follow local guidelines for working in a safe and acceptable manner.

Specifically for **manual extraction**

- Magnetic separation plate or magnet for separating magnetic beads
- benchtop vortex mixer;
- DNase / RNase-free tubes or plates.

Specifically for **automated extraction**
The Kit is compatible with several automatic magnetic separators. The equipment required may vary depending on the instrument used.
For prefilled plates **REF. SBK308,2X32PFI,** specific Rod's tips (to be order separately) are needed according to the automated extractor used.

3. Collection, handling and storage of sample material

3.1 Cultured cells

Cultured cells can be pelleted and then stored at -90 to -65°C until required for RNA purification.

4. Protocol for the isolation of RNA (manual procedure REF SBK308,1X10-SBK308,1X96)

Lyse the sample
Resuspend cell pellet ($5x10^5$ to $1x10^6$ cells) in 350 µL of **STAR BEADS Lysis Buffer 1**. If frozen cell pellets are used as starting material, add 350 µL **STAR BEADS Lysis Buffer 1** to the frozen pellets before thawing.
Add 6 µL of **STAR BEADS Reductant.** Pipet 5–10 times to shear the DNA.

Bind the nucleic acid
Add 350 µL of **isopropanol** and 30 µL of **STAR BEADS Magnetic Beads** to the lysate sample.
Mix for 5 min at room temperature. Briefly centrifuge the sample and after 5 minutes of separation on the magnetic support remove the supernatant.

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

DNA digestion
Add 275 µL of **STAR BEADS DNase Reaction Buffer** and 25 µL of **STAR BEADS DNase working solution** (prepared as described in section 2.2) to the magnetic beads. Incubate at room temperature for 15 minutes.

Rebinding
Add 350 µL of **isopropanol.** Mix for 5 min at room temperature.
Briefly centrifuge the sample and after 5 minutes of separation on the magnetic support remove the supernatant.

Wash magnetic beads
Add 600 µL of **STAR BEADS Washing Buffer 1G** and mix well by vortexing and inverting the tube up and down several times for at least 2 minutes.

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

Wash magnetic beads

- Add 900 µL of **STAR BEADS Washing Buffer 2G** and mix well by vortexing and inverting the tube up and down several times for at least 2 minutes. Briefly centrifuge the sample and after 2-3 minutes of separation on the magnetic support remove the supernatant.

Wash magnetic beads

- Add 900 µL of **STAR BEADS Washing Buffer 2G** and mix well by vortexing and inverting the tube up and down several times for at least 2 minutes. Briefly centrifuge the sample and after 2-3 minutes of separation on the magnetic support remove the supernatant.

Dry magnetic beads

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

Elute highly pure RNA

Add **100 µL STAR BEADS Elution Buffer** and mix well by vortexing. Do not use the pipette to mix the magnetic beads. It is essential to cover the magnetic beads completely with elution buffer during this step.
Incubate at RT for 5 minutes and mix by vortexing several times.

Collect nucleic acids

Separate 5-10 min on the magnetic support and transfer supernatant containing the eluted RNA into a new DNase/RNase free plate/tube.

Note:

In some cases, traces of STAR BEADS Magnetic Beads may be left in the eluate. An additional separation step either using centrifugation or a magnetic separator is recommended to separate any traces of particles.

5. Protocol for the isolation of RNA (automated procedure, with bottle format REF SBK308,1X10 – SBK308,1X96)

5.1. Automated extraction with STAR BEADS RNA Extraction kit, bottle format REF SBK308,1X10 – SBK308,1X96 in combination with Allsheng Auto-Pure Mini, Allsheng Auto-Pure32 A, BIOER GenePure Pro NPA-32P, BIGFISH BFEX 32

- 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	Isopropanol	350 µL
	STAR BEADS Magnetic Beads REF. SBB188	30 µL
2-8	STAR BEADS DNase working solution*	25 µL
	STAR BEADS DNase Reaction Buffer	275 µL
3-9	STAR BEADS Washing Buffer 1 REF. SBW284	600 µL
4-10	STAR BEADS Washing Buffer 2 REF. SBW285	900 µL
5-11	STAR BEADS Washing Buffer 2 REF. SBW285	900 µL
6-12	STAR BEADS Elution Buffer REF. SBEB228	100 µL

(*prepared as described in section 2.2)

- Turn on the extractor.
- Make sure you have downloaded the correct protocols ** on the instrument. To complete the extraction, two protocols are needed (**SBRNA1** for the first step and **SBRNA2** for the second step).
  IMPORTANT: In BIGFISH BFEX 32 extractor a specific set-up is needed before proceeding with the extraction.
  From the central screen click on "System Setting" and then click on "Motion parameter Setting"; enter the password (the default password is 123456), and set the "tip Position" to 73 and then click on OK.

- Insert a new **Rod's Tip** (to be ordered separately) into the instrument (be sure 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 STAR BEADS Extraction Plates used (2 Rod's Tips for each Extraction Plate).
- Place the Extraction Plate in the instrument, in the same position where the Rod's tip was previously inserted and with the labels attached to the plates facing the operator.
- **Lyse the sample:** Resuspend cell pellet ($5x10^5$ to $1x10^6$ cells) in 350 µL of **STAR BEADS Lysis Buffer 1**. If frozen cell pellets are used as starting material, add 350 µL **STAR BEADS Lysis Buffer 1** to the frozen pellets before thawing.
- Add 6 µL of **STAR BEADS Reductant.** Pipet 5–10 times to shear the DNA.
- Add the **whole sample** to the relevant wells in column 1/7 of the Extraction Plate.
- Place the **STAR BEADS RNA Extraction Plate** in the instrument, in the same position where the Rod's tip was previously inserted and with the labels attached to the plates facing the operator.
- Choose the program **SBRNA1** and press "Run".
- After the run is over, remove the Extraction Plate from the instrument and add **350 µL of Isopropanol** in column 2/8 of the Extraction Plate.
- Place the Extraction Plate in the instrument, in the same position where it was during the first step.
- Choose the program **SBRNA2** and press "Run".
- After the second extraction session is over, remove the Extraction Plate from the instrument, recover the RNA from columns 6 and 12 and proceed with the downstream applications.

  Note:

  In some cases, traces of STAR BEADS Magnetic Beads may be left in the eluate. An additional separation step either using centrifugation or a magnetic separator is recommended to separate any traces of particles.

* 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-rna-extraction-kit-bottle-prefilled-plate-formats/

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

6. Protocol for the isolation of RNA (automated procedure with prefilled format REF. SBK308,2X32PFI)

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 RNA Extraction Kit, Prefilled Plates REF SBK308,2X32PFI in combination with Allsheng Auto-Pure Mini, Allsheng Auto-Pure32 A, BIOER GenePure Pro NPA-32P, BIGFISH BFEX 32.

- 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 RNA Extraction Plate**. Orient the plate so that the label faces the operator, with the wells of column 1 on the left side.
- Turn on the extractor.
- Make sure you have downloaded the correct protocols ** on the instrument. To complete the extraction, two protocols are needed (**SBRNA1** for the first step and **SBRNA2** for the second step).
  IMPORTANT: In BIGFISH BFEX 32 extractor a specific set-up is needed before proceeding with the extraction.
  From the central screen click on "System Setting" and then click on "Motion parameter Setting"; enter the password (the default password is 123456), and set the "tip Position" to 73 and then click on OK.
- Insert a new **Rod's Tip** (to be ordered separately) into the instrument (Be sure 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 STAR BEADS RNA Extraction Plates used (2 Rod's Tips for each Extraction Plate).

- Add 275 µL of **STAR BEADS DNase Reaction Buffer** and 25 µL of **STAR BEADS DNase working solution** (prepared as described in section 2.2) to Column 2/8.
- **Lyse the sample:** Resuspend cell pellet ($5x10^5$ to $1x10^6$ cells) in 350 µL of **STAR BEADS Lysis Buffer 1**. If frozen cell pellets are used as starting material, add 350 uL **STAR BEADS Lysis Buffer 1** to the frozen pellets before thawing.
- Add 6 µL of **STAR BEADS Reductant.** Pipet 5–10 times to shear the DNA.
- Add the **whole sample** to the relevant wells in column 1/7 of the Extraction Plate.
- Place the **STAR BEADS RNA Extraction Plate** in the instrument, in the same position where the Rod's tip was previously inserted and with the labels attached to the plates facing the operator.
- Choose the program **SBRNA1** and press "Run".
- After the run is over, remove the Extraction Plate from the instrument and add **350 µL of Isopropanol** in column 2/8 of the Extraction Plate.
- Place the Extraction Plate in the instrument, in the same position where it was during the first step.
- Choose the program **SBRNA2** and press "Run".
- After the second extraction session is over, remove the **STAR BEADS RNA Extraction Plate** from the instrument, recover the purified RNA from columns 6 and 12 and proceed with the downstream applications.

  Note: In some cases, traces of STAR BEADS Magnetic Beads may be left in the eluate. An additional separation step either using centrifugation or a magnetic separator is recommended to separate any traces of particles.

* 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-rna-extraction-kit-bottle-prefilled-plate-formats/

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

7. 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\
- Biological samples 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 Buffer 1, STAR BEADS Magnetic Beads, STAR BEADS Washing Buffer 1G, STAR BEADS RNA Sample Plate, STAR BEADS RNA Washing 1 Plate and STAR BEADS RNA 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 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.

Contact and technical support information:
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8. Ordering information

PRODUCT	ORDER - N	COMPATIBLE EXTRACTORS	UNIT SIZE
STAR BEADS RNA Extraction Kit	SBK308,1X10	Allsheng Auto-Pure 96 Allsheng Auto-Pure Mini, Allsheng Auto-Pure 32 A, BIOER GenePure Pro NPA-32P, BIGFISH BFEX 32	10 preps
	SBK308,1X96		96 preps
	SBK308,2X32PFI	Allsheng Auto-Pure Mini, Allsheng Auto-Pure 32 A, BIOER GenePure Pro NPA-32P, BIGFISH BFEX 32	64 preps

For further information

Visit: **www.cyanagen.com**

Or contact: **technical.support@cyanagen.com**

For orders: **sales@cyanagen.com**

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


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