CYANAGEN
Reagents for Molecular Biology

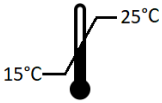
Instruction for Use

STAR BEADS DNA Extraction Kit

REF

SBK307,1X96PFI – SBK307,2X32PFI – SBK307,1X10 – SBK307,1X96

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



Store at +15-25°C

Apr 2024/Rev 00

www.cyanagen.com

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

CYANAGEN
Reagents for Molecular Biology

Table of content

1. General information

1.1. Description

STAR BEADS DNA Extraction Kit provides a fast and efficient purification method to isolate high-quality DNA 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 on the magnetic separation system used. The amount of DNA recovery depends on the type of sample and on 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 2with the addition of STAR BEADS Proteinase K. Binding of nucleic acids to STAR BEADS Magnetic Beads is performed in STAR BEADS Binding Buffer 1. After magnetic separation, the magnetic beads are washed with two washing reagents (STAR BEADS Washing Buffer 1Gand STAR BEADS Washing Buffer 2G) to remove contaminants and salts. Finally, purified DNA is eluted with STAR BEADS Elution Buffer 1 which causes the nucleic acid to detach from the magnetic beads. The resulting high-quality DNA is then ready for use in downstream applications such as RT-PCR, PCR, sequencing, or any other type of enzymatic reaction, or it can be frozen for later use.

1.3. Intended use

STAR BEADS DNA Extraction Kit is intended for use for the extraction of high-quality genomic, bacterial or viral DNA from various body fluids and biological samples such as whole blood, saliva, swabs, cultured mammalian cells, formalin-fixed-paraffin-embedded (FFPE), fresh and frozen tissues

(including mouse tail). In particular, it has been validated by the manufacturer for the extraction of DNA from:

- 200µL of fresh or frozen whole blood, stabilized with either EDTA, citrate or heparin
- 200µL of preserved saliva
- From 10^5 to 10^6 cells of cultured mammalian cells
- 3 - 8 sections (≥ 4 µm) of FFPE
- 2 – 25 mg of Tissue

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 DNA 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.
Whole blood samples collected in blood collection tubes containing EDTA, heparin or sodium citrate anticoagulants can be used. The Kit is not intended for use with samples that have been collected in other types of blood collection tubes.
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 for subsequent downstream analysis such as PCR, qPCR or sequencing to obtain information about the DNA in the sample.

1.4. Analytical performance

1.4.1. Precision

Precision testing	CV% on DNA yield	CV% on A260/280
Intra-assay variability	• 8.30% (SBK261,2X32PFI) • 17.26% (SBK265,1X96) • 4.73% (SBK287,1X96PFI)	• 1.60%(SBK261,2X32PFI) • 1.77%(SBK265,1X96) • 1.07%(SBK287,1X96PFI)
Inter-assay /inter- day variability	8.22%	1.59%
Inter-instrument variability	17.27%*	0.99%
Inter-format variability	20.18%	5.97%

Table 1. Precision evaluation of STAR BEADS DNA Extraction Kit. gDNA was extracted from 200 µL of frozen whole blood in up to 6 replicates per run. Inter-assay and inter-day variability were evaluated in three independent runs performed in two different days. Inter-format variability was evaluated for all the formats of the Kit on the compatible extractors with their dedicated scripts/manual procedure. *The inter-instrument variability was calculated excluding BigFish BFEX 32 extractor, since it is well known that it provides a lower performance. The level of performance, anyway, is considered acceptable, even with a 50% decrease of yield from the average results obtained from the other instruments.

1.4.2. Analytical specificity

The **Analytical Specificity** was evaluated together with the **Cross-contamination** by extraction of 8 frozen blood samples (as positive) and 8 water samples (as negative), tested alternatively on all the pre-filled formats on the relative compatible extractor with the dedicated scripts. For the evaluation, a PCR test was chosen, since the use of the spectrophotometer for the quantification of DNA could give false negative, due to the low sensitivity (10ng/µL, corresponding to a final quantity of 1 µg in the eluate). The extracted DNA were amplified using TaqMan™ Fast Advanced Master Mix (Thermo Scientific, Cat. N. 4444557) and TaqMan primer/probe set for Human RNase P (IDT). All the positive samples resulted positive and all the negative samples resulted to be negative (Ct≥40), affording a 100% analytical specificity.

1.4.3. Linearity

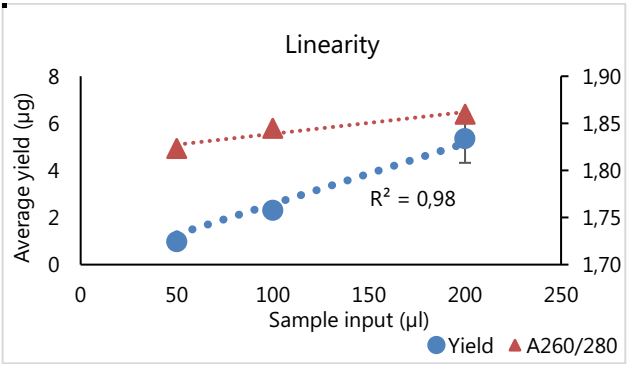


Figure1. Linearity on gDNA extraction with STAR BEADS DNA Extraction Kit
Yield of genomic DNA extracted from different input volumes of whole blood using 50, 100 and 200 µL as sample input demonstrates a linear correlation between the input volume and DNA yield with a good R2 = 0,98. Graph shows average values from two readings. Data are mean ±SD (n=3).

1.5. Performance in clinical downstream assays

1.5.1. SNP Genotyping on gDNA extracted with the STAR BEADS DNA Extraction Kit vs the Reference Extraction Kits

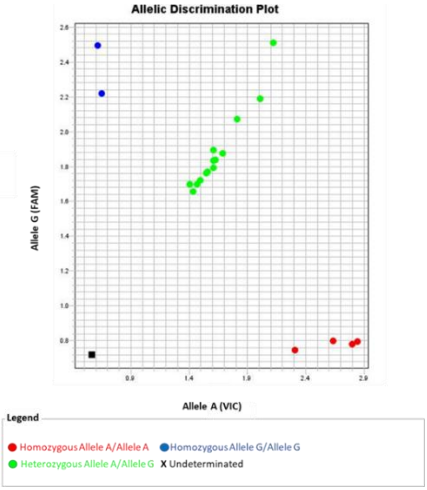


Figure 2. Allelic discrimination plot for the BCL11A rs1123573 SNP assay.
Genotypes for the rs1123573 SNP using gDNA extracted from 200 µL of whole blood from 5 donors and 200 µL of saliva from 5 donors, with STAR BEADS DNA Extraction Kit in parallel respectively with Reference Extraction Kit 1 and Reference Extraction Kit 2. Allele A is shown in red and allele G is shown in blue. Lime green represents the samples heterozygous for the two alleles. Courtesy of U.O. Medical Genetics – Sant'Orsola Malpighi Hospital, Bologna, Italy

	Reference Extraction Kit 1				
STAR BEADS DNA Extraction Kit	**rs1123573**	A/A	A/G	G/G	Tot
	A/A	2	0	0	2
	A/G	0	3	0	3
	G/G	0	0	0	0
	Tot	2	3	0	5

Table 2. Agreement between genotypes of BCL11A rs1123573 SNP resulted from TaqMan assay on the **blood samples** extracted either with STAR BEADS DNA Extraction Kit and Reference Extraction Kit 1. A 100% agreement is obtained for all the samples analysed. Courtesy of U.O. Medical Genetics – Sant'Orsola Malpighi Hospital, Bologna, Italy

	Reference Extraction Kit 2				
STAR BEADS DNA Extraction Kit	**rs1123573**	A/A	A/G	G/G	Tot
	A/A	0	0	0	0
	A/G	0	4	0	4
	G/G	0	0	1	1
	Tot	0	4	1	5

Table 3. Agreement between genotypes of BCL11A rs1123573 SNP resulted from TaqMan assay on the **saliva samples** extracted either with STAR BEADS DNA Extraction Kit and Reference extraction Kit 2. A 100% agreement is obtained for all the samples analysed. Courtesy of U.O. Medical Genetics – Sant'Orsola Malpighi Hospital, Bologna, Italy

1.5.2. NGS on gDNA extracted with STAR BEADS DNA Extraction Kit vs a Reference Extraction Kit.

Blood sample	Extraction kit	NGS platform	*MET* chr7 g.116435768C>T c.468C>T p.Asp156=	*APC* chr5 g.112173836 delAT, c.2547_2548delTA p.Asp849GlufsTer62
bl_1	Reference Extraction Kit	Ion Torrent™ Sequencing System	-	+
	STAR BEADS DNA Extraction kit	MiSeq™ Sequencing System	-	+
bl_2	Reference Extraction Kit	Ion Torrent™ Sequencing System	+	-
	STAR BEADS DNA Extraction kit	MiSeq™ Sequencing System	+	-

Table 4. NGS of a custom target gene library used in oncologic diagnostics. gDNA was isolated from 200 µL of whole blood from 2 subjects in parallel with STAR BEADS DNA Extraction Kit and a Reference Extraction Kit. The results obtained for NGS using gDNA extracted with either the STAR BEADS DNA Extraction Kit or the Reference Extraction Kit were in agreement for all the samples analysed (100% agreement). Courtesy of U.O. Medical Genetics – Sant'Orsola Malpighi Hospital, Bologna, Italy

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 SBK307,1X10 SBK307,1X96. 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) SBK307, 1X10 (sample size)	Kit size (96 preps) SBK307, 1X96
STAR BEADS Lysis Buffer 2*	SBLB282		1,5 mL	10 mL
STAR BEADS Magnetic Beads	SBB188		0,75 mL	6 mL
STAR BEADS Binding Buffer 1	SBBB283		3,5 mL	30 mL
STAR BEADS Washing Buffer 1G*	SBWB284		18 mL	160 mL
STAR BEADS Washing Buffer 2G	SBWB285		9 mL	80 mL
STAR BEADS Elution Buffer 1	SBEB286	None	1,5 mL	12 mL
STAR BEADS Proteinase K	SBK263,0750		0,75 mL	3X 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.

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

Prefilled plate format: REF SBK307,1X96PFI. Use in combination with Allsheng Auto-Pure 96.

Components	REF	GHS
STAR BEADS DNA Sample Plate (1) – Prefilled with STAR BEADS Lysis Buffer 2* - SBLB282 (80 µL/well)	**SBSP288,1X96PFI**	
STAR BEADS Magnetic Beads Plate (1) - Prefilled with STAR BEADS Magnetic Beads - SBB188 in water (500 µL/well)	**SBMBP289,1X96PFI**	
STAR BEADS DNA Binding Plate (1) – Prefilled with STAR BEADS Binding Buffer 1 - SBBB283 (300 µL/well)	**SBBP293,1X96PFI**	
STAR BEADS DNA Washing 1 Plate (2) – Prefilled with STAR BEADS Washing Buffer 1G* - SBWB284 (800 µL/well)	**SBWP290,1X96PFI**	
STAR BEADS DNA Washing 2 Plate (1) – Prefilled with STAR BEADS Washing Buffer 2G- SBWB285 (800 µL/well)	**SBWP291,1X96PFI**	
STAR BEADS DNA Elution Plate (1) – Prefilled with STAR BEADS Elution Buffer 1 - SBEB286 (100 µL/well)	**SBEP292,1X96PFI**	**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.

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

Prefilled All Inclusive format: REF SBK307,2X32PFI. 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 DNA Extraction Plate (4) **Prefilled with:** Lines 1 and 7: STAR BEADS Lysis Buffer 2* SBLB282 (80 µL/well) - Lines 2 and 8: - STAR BEADS Magnetic Beads SBB188 in water (500 µL/well) - Lines 3 and 9: STAR BEADS Washing Buffer 1G* SBWB284 (800 µL/well) Lines 4 and 10: STAR BEADS Washing Buffer 1G* SBWB284 (800 µL/well) Lines 5 and 11- STAR BEADS Washing Buffer 2G SBWB285 (800 µL/well) - Lines 6 and 12: STAR BEADS DNA Elution Buffer (100 µL/well)	**SBK262,1X16PFI**	
STAR BEADS Binding Buffer 1, 30 mL	**SBBB283,0030**	
STAR BEADS Proteinase K (2x 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.

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. 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.
Do not store the pre-filled plates upside down, but with the side closed with the aluminum sheet facing upwards.

2.3. DNA eluates stability

For short-term storage of up to few hours, purified DNA can be stored at room temperature. For long-term storage, it is recommended to store at 2-8ºC or -20°C. DNA purified with the STAR BEADS DNA Extraction Kit is stable for at least 2 years at 2-8ºC or 10 years at -20ºC.

2.4. Required materials to be supplied by the user

- Micropipettes suitable for pipetting 10-20 µL, 150 µL, 300 µL, 500 µL;
- Disposable tips without 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.
- optional: rotating tube mixer for liquid blood samples

Specifically for **manual extraction**

- Magnetic separation plate or magnet for separating magnetic beads
- benchtop vortex mixer;
- heating block;
- 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. SBK307,2X32PFI,** 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. Whole blood

Use an aliquot of 200µL of fresh or frozen whole blood samples.

Note:

- Frozen samples shall be thawed before processing. The yield and purity of extracted gDNA is ensured from blood samples subjected to up to 3 freeze-thaw cycles.

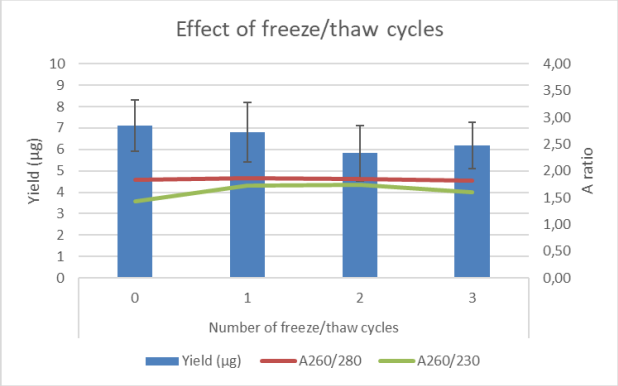


Figure 3. Effects of freezing and thawing blood samples. STAR BEADS DNA Extraction Kit was used to purify genomic DNA from 200 µL of EDTA-treated blood frozen and thawed up to 3 times. Each bar on the graph represents the results from 4 replicates (mean ± standard deviation).

- All blood samples shall be thoroughly mixed before use.
- Avoid clot material (if any) when transferring blood to the incubation tube/well into the prefilled plate.
- This kit has been tested with human whole blood samples collected in EDTA, sodium citrate or heparin tubes, in terms of extraction performance and PCR inhibition. The validated blood collection tubes are shown in Table 5.

Performance of the chemistry of the kit cannot be guaranteed with other types of blood collection tubes. It is the user's sole responsibility to use and validate the Kit in combination with other types of blood collection tubes.

Collection tube	REF.	manufacturer	Avg DNA yield (µg)
BD Vacutainer™ NH 68 I.U. (Sodium Herapin)	BD 367869	BD	4.9
BD Vacutainer™ 9NC 0.105M Buff. Na 3 Citrate	BD 366575	BD	3.7
BD Vacutainer™ K2E (EDTA) 10.8mg	BD 367864	BD	4.6
BD Vacutainer™ K2E (EDTA) 10mL	BD 367525	BD	3.7
BD Vacutainer™ K3EDTA	BD 368857	BD	3.8

Table 5. Compatible collection tubes with anticoagulants. Genomic DNA was purified from 200 µl blood samples from 2 healthy donors. Average yield and purity calculated on the mean of at least four extraction replicates for each tube. CV on DNA yield is ≤ 2% and the absence of PCR inhibition has been demonstrated with (ΔCt (undiluted-1:10 diluted eluted gDNA) < 0.4)

- For blood specimen collection and storage, the collection tube manufacturer's instructions are to be followed.
  Follow the ISO 20186-2:2019(E) for storage guidelines, i.e. for examinations requiring HMW DNA, the specimen should be stored at room temperature for not longer than one day or at 2 °C to 8 °C for not longer than three days. For longer storage, the specimen should be kept at −20 °C for not longer than 1 month, or at −70 °C or below for even longer storage; for the examination of DNA variants not requiring HMW DNA examinations, the specimen should be stored at room temperature for up to 3 days or at 2 °C to 8 °C for up to 7 days. For longer storage, the specimen should be kept at −20 °C for up to 3 months or at −70 °C or below for even longer storage.

- Total yield of genomic DNA from whole blood samples depends on the sample volume and the number of white blood cells (WBC)/mL. WBC count in the whole blood sample decreases during storage. Prolonged storage of the samples may cause a poor yield of the DNA after extraction. Also hemolysis and hyperlipidemia may impact DNA yield.

- WBC count on each sample prior to purification of DNA is recommended to ensure the sample falls within the typical range for a normal healthy donor (4.0 - 10 × 10^6 cells/mL).

3.2. Saliva

Use up to 300 µL aliquot of human saliva stored according to the collection tube provider's instructions.

Note:

- The total yield of genomic DNA depends on the cellular material present in the stabilized saliva specimen. The amount of cellular material in the saliva is dependent on donor, collection technique, and donor behavior before collection. To maximize the yield, please follow the instruction of the collection tube provider.
- This kit has been tested with preserved saliva samples collected in Genotek ORAgene DNA OG-575 collection system. Performance of the chemistry cannot be guaranteed with other types of saliva collection systems. It is the user's sole responsibility to use and validate the Kit in combination with other types of saliva collection systems.

3.4 Cultured cells

Collect cells according to the appropriate culture conditions. Resuspend cell pellet (1x10^5 to 1x10^6 cells) in 200 µL of PBS and proceed following the Protocol in sections 4-5-6 (manual/automated procedure).

3.5 Other Biological Fluids (serum, plasma, urine)

Use up to 300 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.

3.6 Tissues

In a clean empty tube add 200 µL of **STAR BEADS Tissue Lysis Buffer** (to be order separately, cod. SBLB309). Prepare a section of 5 - 25 mg of fresh or frozen tissues or small-size organisms such as insects and add to the tube with **STAR BEADS Tissue Lysis Buffer**.

IMPORTANT! In case of frozen tissue, do not allow the sample to thaw before the lysis step.

Add 25 µL of **STAR BEADS Proteinase K** (to be order separately, cod. SBK263)**.** Vortex the sample for 10 seconds and briefly centrifuge the tube. Incubate at 56°C overnight.

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 5600 RPM for 5 min at room temperature to precipitate possible residue to the bottom of the microcentrifuge tube, and collect the supernatant.

Continue extraction following the Protocol in sections 4-5-6 (manual/automated procedure) starting from Binding step.

3.7 FFPE tissues

This protocol is suitable for genomic DNA extraction from at least 3 sections (≥ 4 µm) 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 (≥ 4 µm) of tissue per sample with 300 µL of **STAR BEADS Lysis Buffer** (to be ordered separately, cod. SBPLB254) in a new 1.5 mL microcentrifuge tube (not provided)
  Note: if the specimen is on a glass microscope slide, once deparaffinized, dispense 150 µL of **STAR BEADS Lysis Buffer** on the slice and then use another 150 µL to include any remains and collect all the tissue from the slide
- 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. 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 µL of the supernatant.
- Continue extraction following the Protocol in sections 4-5-6 (manual/automated procedure).

3.8 Swabs

For dry swab, place the dry swab in 400 µL of **STAR BEADS Lysis Buffer**. Add 20 µL 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 µL .
Continue extraction following the Protocol in sections 4-5-6 (manual/automated procedure).
For swab in Universal Transport Media or other preservation solution, incubate the swab for 30 minutes with gentle shaking to release sample material. Use a 200 µL aliquote to proceed with the Protocol in sections 4-5-6 (manual/automated procedure).

4. Protocol for the isolation of DNA (manual procedure REF SBK307,1X10 SBK307,1X96)

Lyse the sample

- In a clean empty tube, add in the order as follows:
  80 µL of **STAR BEADS Lysis Buffer 2**
  Up to 300 µL of **sample**
- In case it has not been previously added during sample preparation described in section 3, add 20 µL of **STAR BEADS Proteinase K.**
- Vigorously vortex for 30 seconds to completely mix STAR BEADS Lysis Buffer 2, sample and STAR BEADS Proteinase K.
- Incubate for 10 minutes at room temperature, vortexing every 2 minutes.

Note: optimization may be required for incubation time and incubation temperature, depending on the sample type.

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

Bind the nucleic acid

- Add 300 µL **of STAR BEADS Binding Buffer 1** and 50 µL of **STAR BEADS Magnetic Beads** to the lysate sample.
- Vigorously vortex 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 800 µL **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 800 µL **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 800 µL **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 DNA

- Add **100 µL STAR BEADS Elution Buffer 1** 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 55°C for 5 minutes in a heating block and mix by vortexing several times.

Collect nucleic acids

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

Note:

In some cases, traces of STAR BEADS Magnetic Beads may be left in the eluate. Although such particles usually do not interfere with PCR or most downstream applications, 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 DNA (automated procedure, with bottle format REF SBK307,1X10 – SBK307,1X96)

5.1. Automated extraction with STAR BEADS DNA Extraction kit, bottle format REF SBK307,1X10 – SBK307,1X96 in combination with Allsheng AutoPure 96,

- Set up the plates using standard 96 deep well plates and 96 Tip comb. 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 Buffer 2 REF. SBLB282	80 µL
Magnetic Beads Plate	STAR BEADS Magnetic Beads REF. SBB188	50 µL
	Ultrapure water	450 µL
Washing 1 Plate	STAR BEADS Washing Buffer 1G REF. SBW284	800 µL
Washing 2 Plate	STAR BEADS Washing Buffer 1G REF. SBW284	800 µL
Washing 3 Plate	STAR BEADS Washing Buffer 2G REF. SBW285	800 µL
Elution Plate	STAR BEADS Elution Buffer 1 REF. SBEB286	100 µL

- Add up to **300 µL of sample** 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.
- Add the appropriate Extraction Controls* to the relevant wells of the Sample Plate.
- Turn on the extractor.

- Make sure you have downloaded the correct protocols ** on the instrument. To complete the extraction, two protocols are needed (**SBDNA1** for the first step, and **SBDNA2** for the second step) **IMPORTANT** for Tissues extraction only the protocol SBDNA2 is needed.
- 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
Magnetic Beads	3
Washing 1 Plate	4
Washing 2 Plate	5
Washing 3 Plate	6
Elution Plate	8

- Choose the program **SBDNA1** and press "Run". After the run is over, remove the Sample Plate from the instrument.
  IMPORTANT for Tissues extraction: skip this step and continue from the next step.
- Add **300 µL of STAR BEADS Binding Buffer 1** to the relevant wells of the Sample Plate.
- Place the Sample Plate in the position 2 of the instrument, in the same position where it was during the first step.
- Choose the program **SBDNA2** and press "Run".
- After the extraction session is complete, remove the Elution plate from position 8 of the instrument and proceed with downstream applications.
  Note:
  In some cases, traces of STAR BEADS Magnetic Beads may be left in the eluate. Although such particles usually do not interfere with PCR or most downstream applications, 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-dna-extraction-kit-bottle-prefilled-plate-formats-ruo/

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

5.2. Automated extraction with STAR BEADS DNA Extraction kit, bottle format REF SBK307,1X10 – SBK307,1X96 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	STAR BEADS Genomic Lysis 2 REF. SBLB282	80 µL
2-8	STAR BEADS Magnetic Beads REF. SBB188	50 µL
	Ultrapure water	450 µL
3-9	STAR BEADS Washing Buffer 1G REF. SBW284	800 µL
4-10	STAR BEADS Washing Buffer 1G REF. SBW284	800 µL
5-11	STAR BEADS Washing Buffer 2G REF. SBW285	800 µL
6-12	STAR BEADS Elution Buffer 1 REF. SBEB286	100 µL

- Add up to **300 µL of sample** 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.
- Add the appropriate Extraction Controls * to the appropriate wells of the Extraction Plate.
- Turn on the extractor.

- Make sure you have downloaded the correct protocols ** on the instrument. To complete the extraction, two protocols are needed (SBDNA1 for the first step and SBDNA2 for the second step).
  IMPORTANT for Tissues extraction only the protocol SBDNA2 is needed.
  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.
- Choose the program **SBDNA1** and press "Run". After the run is over, remove the Extraction Plate from the instrument.
  IMPORTANT for Tissues extraction, skip this step and continue from the next step.
- Add **300 µL of STAR BEADS Binding Buffer 1** in column 1/7 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 **SBDNA2** and press "Run".
- After the second extraction session is over, remove the Extraction Plate from the instrument, recover the DNA 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. Although such particles usually do not interfere with PCR

or most downstream applications, 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-dna-extraction-kit-bottle-prefilled-plate-formats-ruo/

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

6. Protocol for the isolation of DNA (automated procedure with prefilled format REF. SBK307,2X32PFI – SBK307,1X96PFI)

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 DNA Extraction Kit, Prefilled Plates REF SBK261,2X32PFI 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 DNA Extraction Plate**. Orient the plate so that the label faces the operator, with the wells of column 1 on the left side.
- Add up to **300 µL of sample** to the relevant wells in column 1/7 of the **STAR BEADS DNA 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 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 DNA Extraction Plate**.
- Turn on the extractor.
- Make sure you have downloaded the correct protocols ** on the instrument. To complete the extraction, two protocols are needed (**SBDNA1** for the first step and **SBDNA2** for the second step).

IMPORTANT for Tissues extraction only the protocol SBDNA2 is needed.

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 Genomic Extraction Plates used (2 Rod's Tips for each Extraction Plate).
- Place the **STAR BEADS DNA 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 **SBDNA1** and press "Run". After the run is over, remove the **STAR BEADS DNA Extraction Plate** from the instrument.

  IMPORTANT for Tissues extraction, skip this step and continue from the next step.
- Add **300 µL of STAR BEADS Binding Buffer 1** to the relevant wells in column 1/7 of the **STAR BEADS DNA Extraction Plate**.
- Place the **STAR BEADS DNA Extraction Plate** in the instrument, in the same position where it was during the first step and with the labels attached to the plates facing the operator.
- Choose the program **SBDNA2** and press "Run".
- After the second extraction session is over, remove the **STAR BEADS DNA Extraction Plate** from the instrument, recover the purified gDNA 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. Although such particles usually do not interfere with PCR

or most downstream applications, 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-dna-extraction-kit-bottle-prefilled-plate-formats-ruo/

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

6.3. Automated extraction with STAR BEADS DNA Extraction kit, Prefilled Plates format REF. SBK307,1X96PFI in combination with Allsheng Auto-Pure 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 DNA Sample Plate**.
- Add up to **300 µL** of sample to the relevant wells of the **STAR BEADS DNA 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 in 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 DNA Sample Plate**.
- Turn on the extractor.
- Make sure you have downloaded the correct **extraction protocol** ** to the instrument. To complete the extraction, two protocols are

needed (**SBDNA1** for the first step and **SBDNA2** for the second step).

IMPORTANT for Tissues extraction only the protocol SBDNA2 is needed.

- Remove the aluminum foil from the **STAR BEADS Magnetic Beads Plate**, **STAR BEADS DNA Washing 1 Plate (X2)**, **STAR BEADS DNA Washing 2 Plate, STAR BEADS DNA Elution Plate** and 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 DNA Sample Plate	2 (skip this for tissues extraction)
STAR BEADS Magnetic Beads Plate	3
STAR BEADS DNA Washing 1 Plate	4
STAR BEADS DNA Washing 1 Plate	5
STAR BEADS DNA Washing 2 Plate	6
STAR BEADS DNA Elution Plate	8

- Choose the program **SBDNA1** and press "Run". After the run is over, remove the **STAR BEADS Genomic Sample Plate** from the instrument, take the **STAR BEADS Genomic Binding Plate** and remove the aluminum foil, then transfer **300 µL of STAR BEADS Binding Buffer 1** to the relevant wells in the **STAR BEADS DNA Sample Plate**.

  IMPORTANT for Tissues extraction, skip this step and continue from the next taking the STAR BEADS Genomic Binding Plate and remove the aluminum foil, then transfer 300 µL of STAR BEADS Binding Buffer 1 to the relevant wells in the STAR BEADS Genomic Sample Plate.
- Place the **STAR BEADS DNA Sample Plate** in the instrument, in position 2 in the same position where it was during the first step.
- Choose the program **SBDNA2** and press "Run".

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

  Note:
  In some cases, traces of STAR BEADS Magnetic Beads may be left in the eluate. Although such particles usually do not interfere with PCR or most downstream applications, 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-dna-extraction-kit-bottle-prefilled-plate-formats-ruo/

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

7. Troubleshooting

For Bottle format:

Problem	Possible Cause	comments/suggestions
Low or inconsistent yield	Low concentration of white blood cells in sample	The DNA yield depends on the number of white blood cells per sample. Blood samples with low white blood cell count yield low DNA amounts.
	Low concentration	Some saliva samples contain very little amount of DNA. This varies from individual to individual based on numerous variables.

	of cells in saliva sample	Ensure that saliva is collected strictly following the manufacturer's instructions.
	Degraded DNA in whole blood samples	Blood that has undergone multiple freeze-thaw cycles may have degraded DNA. Use samples that have been collected and stored under the conditions listed in Section 3.1.
	Incomplete sample lysis	Inhomogeneous blood sample or blood clots within the sample: Make sure that blood samples are collected following the instructions of the manufacturer of the blood collection tube. Make sure that only blood which can be easily transferred by pipetting is used as sample material. If necessary, homogenize the blood sample before use. Sample not thoroughly mixed with Proteinase K and lysis buffer. The mixture has to be vortexed vigorously immediately after addition of STAR BEADS Lysis Buffer 2. Proteinase K digestion not optimal. Never add Proteinase K directly to the STAR BEADS Lysis Buffer 2. For blood samples, if Proteinase K was not added, the resulting blood sample will be red. Proteinase K-treated samples turn dark red/brownish, which can be used as a visual indicator that Proteinase K was added to the sample.
	Insufficient elution buffer volume	Beads pellet must be entirely covered with elution buffer. Follow the procedure in section 4.
	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

Magnetic beads carryover	Magnetic separation time too short	In some cases, traces of STAR BEADS Magnetic Beads remain into the eluates which appear brownish. Perform a second magnetic particle capture using the magnetic rack or remove the particles by centrifugation.
Low purity of nucleic acids	Insufficient washing procedure	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 in downstream applications	Ethanol carryover	The magnetic beads should be free from any visible liquid ethanol before the elution step
	Too low concentration	From relative wells, remove up to 40 uL of elution buffer
	DNA degradation	Avoid any nuclease contamination
Low reproducibility of DNA extraction	STAR BEADS Lysis Buffer 2forms salt precipitates if stored below 15°C	Incubate the buffer bottle at 40°C until all of the precipitates are re-dissolved
	STAR BEADS Washing Buffer 1Gforms salt precipitates if stored below 15°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	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 DNA Extraction Plate, STAR BEADS DNA Sample

		Plate, STAR BEADS DNA 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 2X32PFI format make sure to recover the eluates from columns 6 and 12 from the STAR BEADS DNA Extraction Plate. For the 1X96PFI format be sure to retrieve eluates from the STAR BEADS DNA Elution Plate.
	Error in the positioning of the plates in the extractor	For the 2X32PFI format, be sure to insert in the instrument the STAR BEADS DNA Extraction Plate with the label facing the operator. For the 1X96PFI format, make sure to insert the plates in the extractor in the correct position as indicated in section 5.1 and 6.3.
	Low concentration of white blood cells in sample	Same suggestions as for the bottle format
	Low concentration of cells in saliva sample	Same suggestions as for the bottle format
	Degraded DNA in whole blood samples	Same suggestions as for the bottle format
	Incomplete sample lysis	Same suggestions as for the bottle format
Magnetic beads carryover	Magnetic separation time too short	Same suggestions as for the bottle format

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 Buffer 2, STAR BEADS Magnetic Beads, STAR BEADS Washing Buffer 1 G, STAR BEADS DNA Sample Plate, STAR BEADS Washing 1 G Plate and STAR BEADS DNA 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.

9. Ordering information

PRODUCT	ORDER - N	COMPATIBLE EXTRACTORS	UNIT SIZE
STAR BEADS DNA Extraction Kit	SBK307,1X10	Allsheng Auto-Pure 96 Allsheng Auto-Pure Mini, Allsheng Auto-Pure 32 A, BIOER GenePure Pro NPA-32P, BIGFISH BFEX 32	10 preps
	SBK307,1X96		96 preps
	SBK307,1X96PFI	Allsheng Auto-Pure 96	96 preps
	SBK307,2X32PFI	Allsheng Auto-Pure Mini, Allsheng Auto-Pure 32 A, BIOER GenePure Pro NPA-32P, BIGFISH BFEX 32	64 preps

CYANAGEN
Reagents for Molecular Biology

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/


CYANAGEN
Reagents for Molecular Biology