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G2M

oneXtract

One for all Nucleic Acid Extraction Kit

SPINXT

Tissue & body fluids Nucleic acid extraction kit

S. No.	Product Name	Product Code	Pack Size
1.	SpiNXT Tissue & body fluids Nucleic acid extraction kit	G2M131420	50 T
2.	SpiNXT Tissue & body fluids Nucleic acid extraction kit	G2M131420	250 T

Content Table

Intended Use

SpiNXT Tissue & body fluids Nucleic acid extraction kit is an *in-vitro* diagnostic test kit, intended for isolation and purification of nucleic acid from clinical samples such as tissues, product of conception, chorionic villi sample, cell culture pellet, amniotic fluid, bronchial washing, urine, sputum, whole blood, buffy coat, plasma, serum, saliva, pleural fluids, vaginal swabs, cervical swab, oropharyngeal swabs, nasopharyngeal swabs, tissue swabs and bronchoalveolar lavage (BAL).
SpiNXT Tissue & body fluids Nucleic acid extraction kit utilizes silica column based technology and can be processed either manually or automated on most open- ended platforms such as silica column processors.

Intended User

The assay is intended to be performed by a laboratory professional in clinical laboratory.

Test Principle

The kit is based on the selective binding of nucleic acid to silica membrane. The sample is lysed and digested with lysis buffer and protease consequently the nucleic acid is released into the lysate transfer to the spin column. Nuclic acid (DNA/RNA) is adsorbed on the silica membrane, while proteins and other impurities are not adsorbed and washed away. Nucleic acid (DNA/RNA) was finally eluted with low-salt buffer from intended samples.

Summary

The SpiNXT Tissue & Body Fluids Nucleic acid extraction provides a quick and simplest way to purify DNA/RNA for reliable use in downstream applications. This kit is designed to efficiently isolate total nucleic acid of host and pathogens (bacterial/viral/parasitic) from clinical samples such as tissues, product of conception, chorionic villi sample, cell culture pellet, amniotic fluid, bronchial washing, urine, sputum, whole blood, buffy coat, plasma, serum, saliva, pleural fluids, vaginal swabs, cervical swab, oropharyngeal swabs, nasopharyngeal swabs, tissue swabs, bronchoalveolar lavage (BAL). Samples can be fresh or frozen but must be restricted to thaw it more than once. SpiNXT Tissue & Body Fluids Nucleic acid extraction can be used for the purification of DNA/RNA from various types of biological samples but the performance may vary depending upon the sample type.

Materials Required but Not Provided

- Water bath or Heating block
  Micropipettes (Adjustable)
- Disposable barrier (Filter) pipette tips
- 1.5 ml microcentrifuge tubes (RNase and DNase free)
- Table top microcentrifuge
- Molecular biology grade ethanol (96-100%)
- Personal protective equipment (Aprons, disposable gloves, goggles etc).
- Mortar and Pestle
- 1X PBS
- Vortexer
- RNase A Enzyme
- DNase I Enzyme

Storage, Operating Conditions and Stability

- This product can be stored at room temperature (15°C-25°C) for 18 months without any reduction in performance. If precipitate forms in any of the reagents of the kit, warm at 55ºC to dissolve.
- The kit has a shelf life of 18 months from the date of manufacture. The test kit is stable until the expiration date marked on the kit box and/or the packaging of individual components when stored as specified.


Protect from direct sunlight.

Instructions Before Use

- Buffer 1XRT-Wash 1, Buffer 1XRT-Wash 2 are supplied as concentrates. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle and shake thoroughly.
- Buffer 1XRT-Wash 1, Buffer 1XRT-Wash 2 are stable for at least 18 months after the addition of ethanol when stored closed at room temperature (15–25°C).
- After dissolving Proteinase K (20 mg/ml) & Carrier RNA as per the label needs to be stored at be -20ºC in aliquots to avoid repeated freeze thaw cycles.

Sample Preparation Protocol

A). Sample preparation (Tissues/POC/CVS)

- Cut ~25 mg of tissue sample into pieces or grind using mortar and pestle for efficient lysis and transfer it into a 1.5 ml microcentrifuge tube. Add 180 µl Buffer LBTC in case of DNA and 400 µl RLB in case of RNA along with 20 µl proteinase K .
- For DNA purification incubate the mixture at 56°C for 20 min which can be extended up to 1-3 hours depending upon the tissue sample. For RNA purification incubate the sample for 10 min at room temperature and centrifuge the tube at 10,000x*g* for 1 min. Collect the supernantant into a fresh 1.5ml microcentrifuge tube.
- Proceed with step 1 of manual protocol.

B). Sample preparation (Cultured cells/mammalian cells/Amniotic fluid/ Urine/Cervical Swab/Vaginal Swab)

- Centrifuge the appropriate number of cells ($5x10^6$) for 10 min at 5000x*g*. Discard the supernatant in such a way that 20 µl of supernatant remain in it and then resuspend the pellet in 180 µl Buffer LBTC.
- 200 µl of sample was transferred into a 1.5 ml microcentrifuge tube and proceed with step 1 of manual protocol.
- Proceed with step 1 of manual protocol.

In case of Viral DNA/RNA purification, the supernatant 200 µl to be used from manual protocol step 1.

C). Sample preparation (Plasma/Serum)

- Centrifuge 1.5 ml of whole blood at 3000x*g* for 10 min at room temperature. Three layers will be visible.
- Collect approximately 200 µl of upper layer containing plasma/serum carefully into a fresh and sterile 1.5 ml microcentrifuge tube and proceed with step 1 of manual protocol or Automated extraction system step 1.

D). Sample preparation (Saliva/Bronco alveolar lavage [BAL]/pleural fluid/ bronchial washings/ Cerebrospinal fluid/Whole Blood/Bone Marrow/Buffy coat)

- Use 200 µl of above mentioned sample need to be transferred into a 1.5 ml microcentrifuge tube and proceed with step 1 of manual protocol.

E). Sample preparation from Oropharyngeal/ Nasopharyngeal/Tissue swab

- Collect a swab & swirl it for 30 - 60 sec in 1-2 ml of 1X PBS.
- 200 µl of sample need to be transferred into a 1.5 ml microcentrifuge tube and proceed with step 1 of manual protocol.

F) Sample preparation from Sputum.

- 200 µl of inactivated and liquefied sputum was taken into 1.5 ml microcentrifuge tube and proceed with Next step that i.e, addition of Buffer IR given below.
- Add 400 µl of Buffer IR, mix well by vortexing. Centrifuge for 2 mins at 13000x*g*.
- Carefully decant or discard the supernatant by pipetting without disturbing the pellet and add 20 µl of Proteinase K to the pellet.
- After adding Proteinase K break the pellet by finger tapping or vortexing and add 300 µl Buffer 1XRT- TB Lysis mix well and incubate it at 60°C for 20 min.
- After incubation add 300 µl ethanol (96-100%) invert mix and rest for 1 min at RT.
- Proceed with step 3 of manual protocol.

G). Sample preparation (Pus/Abscessic Fluid/Synovial Fluid/ Drain Fluid)

- Allow the sample to thaw at room temperature for 30 min.
- Once thawed transfer 500 µl of sample to 1.5 ml microcentrifuge tube.
- Add 500 µl of Buffer LBTC to the 1.5 ml microcentrifuge tube containing sample, vortex vigorously for 10-15 sec, incubate it at 56°C for 20 min or till the sample digests completely.
- Once the sample is liquefied, transfer 200 µl of sample into 1.5 ml microcentrifuge tube and proceed with step 1 of manual protocol.

SpiNXT Tissue & body fluids nucleic acid extraction kit Platform-Manual

Product Code-G2M131420-(Pack Size -50T), G2M131420-(Pack Size -250T)

Table 1A. Kit Components

Kit Contents	Kit Content Code	Kit Content Quantity	Kit Content Code	Kit Content Quantity
	50 Tests	50 Tests	250 Tests	250 Tests
Buffer 1XRT-Lysis	G2MA3-2922-1	25 ml	G2MA3-2922-2	115 ml
Buffer LBTC	G2MA3-2923-1	15 ml	G2MA3-2923-2	60 ml
Buffer 1XRT-Wash 1	G2MA3-2924-1	12 ml	G2MA3-2924-2	60 ml
Buffer 1XRT-Wash 2	G2MA3-2925-1	12 ml	G2MA3-2925-2	2x30 ml
Buffer AE	G2MA3-2926-1	10 ml	G2MA3-2926-2	30 ml
Proteinase K (20 mg/ml)	G2MA3-2927-1	20 mg	G2MA3-2927-2	100 mg
Protease Dissolve Buffer	G2MA3-2928-1	2 ml	G2MA3-2928-2	6 ml
Carrier RNA	G2MA3-2929-1	50 preps	G2MA3-2929-2	250 preps
Buffer RLB	G2MA3-2930-1	25 ml	G2MA3-2930-2	110 ml
Buffer IR	G2MA3-2931-1	23 ml	G2MA3-2931-2	115 ml
Buffer 1XRT- TB Lysis	G2MA3-2932-1	18 ml	G2MA3-2932-2	80 ml

Table 1B. Consumables

Item Name	Kit Content Quantity for 50 T	Kit Content Quantity for 250 T
Mini column	1 x 50 Nos.	2 x 125 Nos.
Collection Tube	1 x 50 Nos.	2 x 125 Nos.

B. Manual Protocol

1) In 1.5 ml microcentrifuge tube add 450 µl of Buffer-1XRT-Lysis, 20 µl Proteinase K and 2.5 µl Carrier RNA.
2) Transfer 200 µl of sample and mix by vortexing for 15 sec. Keep at room temperature for 10 min.
3) Take out a new Mini column and transfer the sample to the column. Centrifuge at 14,000×*g* for 1 min.
4) Discard the filtrate and place the column back into the collection tube. Add 500 µl Buffer 1XRT Wash 1 to the column. Centrifuge at 14,000×*g* for 1 min.
5) Discard the filtrate and place the column back into the collection tube. Add 500 µl Buffer 1XRT Wash 2 to the column. Centrifuge at 14,000×*g* for 1 min.
6) Repeat step 5.
7) Dry spin the column at 20,000x*g* for 2 min.
8) Transfer the column to a new 1.5 ml microcentrifuge tube.
9) Add 50 µl Buffer AE to the centre of the membrane. Incubate at RT for 3-5 min and Centrifuge at 10,000×*g* for 1 min. (**Note:** Add 100 µl Buffer AE in case of sputum sample).
10) Discard the column and transfer the supernatant containing the purified DNA/RNA to the clean 1.5 ml microcentrifuge tube.

RNase A Digestion (Optional)

If RNA content of the sample is causing an obstruction/interfering in downstream applications of the purified DNA an optional RNase A digestion step may be performed during sample preparation (Add 5 µl RNase A (10 mg/ml) with Buffer 1XRT-Lysis if needed). RNase A digestion is performed during sample preparation to degrade RNA present in the sample and minimize RNA contamination in the purified DNA sample.

DNase I (Optional)

If DNA content of the sample is causing an obstruction/interfering in downstream applications of the purified RNA, an optional DNase I step may be performed after the DNA (As per the manufactures's instructions).

TROUBLESHOOTING GUIDE

A. Poor or low yield of nucleic acid

- **Sample is older -** The yield of the nucleic acid depends upon sample quality, type and volume. Much older Samples allow lysis to occur more readily which eventually leads to degradation of RNA.
- **Elution is incomplete/ Elute contains residual ethanol from the Buffer 1XRT-Wash-** Incubate the column for additional 3-5 min after adding Buffer 1X RT-Wash at room temperature (15-25°C). In order to remove ethanol completely from the final wash with Buffer 1X RT-Wash. Spin down the tubes for longer time to dry the column completely.
- **Ethanol was not added to wash buffer concentrate -** Check whether ethanol is added to wash buffer concentrate as per the instructions on the label before using them.

B. Spin column is clogged

- **Sample too large -** Too much number of cells were placed to the column. Use a much lesser quantity of sample. Clogging can be alleviated by centrifuging the column for a longer time period until whole of the lysate passes through or by increasing the *g* force.

C. RNA is sheared or degraded

- **RNA was handled improperly -** Use sterile, disposable plastic ware, glassware and autoclavable pipettes reserved specifically for RNA work to avoid contamination from shared equipments. Pipetting steps should be taken care of. Change the gloves frequently whenever required.

D. DNA or RNA contamination in downstream applications

- **No DNase treatment/No RNase treatment -** Add DNase I or RNase A after purification of high quality nucleic acid, if needed to remove trace amounts of DNA or RNA to prevent contamination for further downstream processes (Usage as per the manufacturer's instructions).

Limitations

- **Sample Variability:** Different body fluids may contain varying amounts of DNA/RNA, leading to differences in extraction efficiency and yield. Each sample type requires specific preprocessing and the use of appropriate buffers and protocols. Failure to preprocess samples adequately may result in reduced efficiency or failure of DNA/RNA extraction due to potential inhibitors present in certain body fluids.
- **Cross-Contamination:** Improper handling or inadequate cleaning of equipment may lead to cross-contamination between samples, compromising the reliability of the extracted DNA/RNA. Maintaining a contamination-free environment and following proper cleaning protocols are essential to minimize the risk of cross-contamination.
- **Processing Time:** The extraction process can be time-consuming, particularly when dealing with multiple types of body fluids. This can lead to workflow bottlenecks, especially in high-throughput settings. Efficient workflow management and optimization of extraction protocols are necessary to minimize processing time and increase productivity.
- **Operator Skill:** The efficiency of the extraction process may vary depending on the expertise of the operator. Variability in results between different users can occur if operators lack sufficient training or experience. Standardized protocols and regular training sessions can help ensure consistency in results across different operators.

Safety and Precautions

- **Chemical Handling:** Reagent cartridges contain guanidine hydrochloride/guanidine thiocyanate, which can react with bleach to form highly reactive compounds. In case of spillage, clean with laboratory detergent and water.
- **Biological Samples:** Tissues, body fluids, infectious agents, and blood may carry infectious diseases. Ensure all laboratory personnel are familiar with general safety guidelines for chemical usage, storage, and waste disposal. Refer to relevant Safety Data Sheets (SDS) for specific precautions.
- **Personal Protective Equipment (PPE):** Wear appropriate attire, including lab coats, gloves, goggles, and closed-toe shoes to protect against spills, splashes, and inhalation.
- **Ventilation:** Work in well-ventilated areas or use fume hoods to minimize exposure to harmful vapors or inhalation of chemicals. Handle chemical waste in designated fume hoods.

- **Storage:** Store chemicals correctly in designated areas, adhering to guidelines for temperature, compatibility, and segregation.
- **Labeling:** Ensure all containers are clearly labeled with the chemical name, concentration, and hazard warnings to prevent accidents or confusion.
- **Handling:** Employ proper techniques when handling chemicals, such as pouring slowly, avoiding splashing, and refraining from pipetting by mouth. Keep chemical containers closed when not in use.
- **Emergency Equipment:** Familiarize yourself with the location and proper use of safety showers, eyewash stations, fire extinguishers, and spill kits for swift response to emergencies.
- **Training:** All individuals must undergo comprehensive training on chemical handling, emergency procedures, and the use of safety equipment as per regulatory and institutional requirements before handling potentially biohazardous materials.

Symbols for Use in the Labeling	
Symbols	**Definition**
	KEEP AWAY FROM SUNLIGHT
	TEMPERATURE LIMIT
	IN VITRO DIAGNOSTIC MEDICAL DEVICE
	UPWARD
	CONSULT INSTRUCTIONS FOR USE
	BATCH CODE
	CATALOGUE NUMBER
	USE BY DATE
	DATE OF MANUFACTURE
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	CAUTION
	DO NOT USE IF PACKAGE IS DAMAGED
	AUTHORIZED REPRESENTATIVE IN THE EUROPEAN COMMUNITY/ EUROPEAN UNION
	EUROPEAN CONFORMITY

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