EN

REF 1N1000

STAT-NAT® Total DNA/RNA extraction kit

Extraction kit for RNA and DNA isolation from pathogens

2 x 15 mL Lysis Buffer NPL1
2 x 40 mL Binding Buffer NPB2
6 x 15 mL Wash Buffer NPW3
4 x 25 mL Wash Buffer NPW4
2 x 13 mL Elution Buffer NPE5
1 x 400 µg Carrier RNA
1 x 0.5 mL Carrier RNA Buffer
1 x 75 mg Proteinase K
1 x 8 mL Proteinase K Buffer
2 x 1.25 mL Magnetic Beads

Σ =96 extractions

IVD

CE

NOTE: This package insert must be read carefully prior to product use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

INTENDED USE

The STAT-NAT® Total DNA/RNA extraction kit is designed for the isolation of viral RNA and DNA and bacterial DNA from body fluids such as serum or plasma, blood, stool sample suspensions, and swab washes.

PRINCIPLE

The STAT-NAT® Total DNA/RNA extraction kit provides reagents and magnetic beads for isolation of 96 samples. The procedure is based on the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved by incubation with a Lysis Buffer NPL1 containing chaotropic ions supported by Proteinase K digestion. For binding of nucleic acids to the paramagnetic beads, Binding Buffer NPB2 and the Magnetic Beads are added to the lysate. After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Wash Buffers NPW3, NPW4, and 80 % ethanol. Residual ethanol from previous wash steps is removed by air drying. Finally, highly pure pathogen RNA and DNA is eluted with low-salt elution buffer or water. Purified pathogen RNA and DNA can directly be used for downstream applications. The STAT-NAT® Total DNA/RNA extraction kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

REAGENTS

STAT-NAT® Total DNA/RNA extraction kit consists of:

REF	Amount per kit
Lysis Buffer NPL1	2 x 15 mL
Binding Buffer NPB2	2 x 40 mL
Wash Buffer NPW3	6 x 15 mL
Wash Buffer NPW4	4 x 25 mL
Elution Buffer NPE5	2 x 13 mL
Carrier RNA	1 x 400 µg
Carrier RNA Buffer	1 x 0.5 mL
Proteinase K	1 x 75 mg
Proteinase K Buffer	1 x 8 mL
Magnetic Beads	2 x 1.25 mL

SAMPLE

The STAT-NAT® Total DNA/RNA extraction kit can be used to extract DNA and RNA from viruses and bacteria from body fluids such as serum or plasma, blood, stool sample suspensions, and swab washes.

INSTRUMENTATION AND MATERIALS REQUIRED BUT NOT PROVIDED

Reagents: ethanol 80 %.
Magnet for magnetic beads separation: STAT-NAT® Magnetic Rack (REF. 1N1003) or equivalent.
Plastic vial: molecular biology grade plastic vial for the preparation steps. DNase and RNase free plastic vial for store the purified RNA/DNA.
General molecular laboratory equipment: biosafety cabinet (laminar flow hood) for extractions, vortex mixer, variable volume pipettes, sterile disposable plastics.
Personal protective equipment (PPE) as gloves, laboratory coats, safety glasses, facemasks.

WARNINGS AND PRECAUTIONS

- This assay is *in vitro diagnostic* (IVD) use;
- Read all the instructions contained in the kit insert before performing the test;
- Comply with the kit expiration date;
- Do not use reagents from other commercial kits;
- Do not mix up reagents from kits with different Lot Number;
- The MSDS are available at www.sentineldiagnostics.com or at your local supplier;
- Always use personal protective equipment for the individual protection;
- The product must be handled by staff trained in molecular biology techniques, such as nucleic acids extraction, amplification and detection;
- It is required to keep separated the sample extraction area, the reagent preparation area and the amplification/detection area;
- ⚠ **CAUTION** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens[1], Biosafety Level 2[2] or other appropriate biosafety practices[3,4] should be used for materials that contain or are suspected of containing infectious agents.

Hazard Identification:

- Complete product 1N1000

GHS02 GHS07 GHS08

DANGER
Hazard statements:
H226 Flammable liquid and vapour.
H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H412 Harmful to aquatic life with long lasting effects.

Precautionary Statements
P210 Keep away from heat/sparks/open flames/hot surfaces. No smoking.
P261sh Avoid breathing dust/vapours.
P264W Wash with water thoroughly after handling.
P273 Avoid release to the environment.
P280sh Wear protective gloves/eye protection.
P301+312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.
P304+340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P330 Rinse mouth.
P342+311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor.

- **Carrier RNA Buffer**

GHS07

WARNING
Hazard statements:
H302 Harmful if swallowed.
H412 Harmful to aquatic life with long lasting effects.

- Binding Buffer NPB2

GHS02 GHS07

WARNING
Hazard statements:
H226 Flammable liquid and vapour.
H302 Harmful if swallowed.

- Lysis Buffer NPL1

GHS07

WARNING
Hazard statements:
H302 Harmful if swallowed.
H319 Causes serious eye irritation

- Wash Buffer NPW3

GHS02 GHS07

WARNING
Hazard statements:
H226 Flammable liquid and vapour.
H302 Harmful if swallowed.

- Wash Buffer NPW4

GHS02 GHS07

WARNING
Hazard statements:
H226 Flammable liquid and vapour.
H302 Harmful if swallowed.

- Proteinase K

GHS07 GHS08

DANGER
Hazard statements:
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

STAT-NAT® Total DNA/RNA extraction kit Highlights:

STAT-NAT® Total DNA/RNA extraction kit is designed for rapid manual and automated small-scale preparation of viral RNA and DNA and the DNA of microorganisms from various types of clinical samples. The kit is designed for use with STAT-NAT® Magnetic Rack (REF. 1N1003) or other magnetic separation systems. Manual time for the preparation of 96 samples is about 120 minutes. The purified RNA and DNA can be used directly as template for RT-PCR, PCR, or any kind of enzymatic reactions.

INSTRUCTIONS FOR USE

For use of STAT-NAT® Total DNA/RNA extraction kit, the use of the magnetic separator STAT-NAT® Magnetic Rack (REF. 1N1003) is recommended. The kit can also be used with other common magnetic separators.

Storage conditions and preparation of working solutions

Attention:

NPL1, NPB2 and the Carrier RNA Buffer contain chaotropic salt! Wear gloves and goggles!

All components of the STAT-NAT® Total DNA/RNA extraction kit should be stored at room temperature (18– 25 °C). All buffers are delivered ready-to-use.

Before starting any STAT-NAT® Total DNA/RNA extraction kit protocol, prepare the following:

Proteinase K: Before first use of the kit, add 3.35 mL **Proteinase K Buffer (cap with green label)** to each vial of the lyophilized Proteinase K. Dissolved Proteinase K solution should be stored at -20 °C. Once resuspended, the solution is stable up to 6 months at -20 °C.

Carrier RNA: Before first use of the kit, add 500 µL Carrier RNA Buffer to each vial lyophilized Carrier RNA. Store dissolved Carrier RNA solution in aliquots at -20 °C. Once resuspended, the solution is stable up to 6 months at -20 °C.

PROCEDURE

Protocol for the isolation of viral RNA and DNA and microbial DNA from blood, serum, plasma, feces and swab washes

Preparation of sample materials

a) Blood, serum and plasma samples

A sample volume of 100–200 µL blood, serum or plasma is recommended. Do not use higher volumes. When processing less than 200 µL sample adjust with PBS buffer to a final volume of 200 µL.

b) Swab samples

Dry swab: Incubate the swabs in PBS, sodium chloride, or cell culture medium for 30 min with agitation. Then remove the swab pressing it against the walls of the tube to squeeze out most of the liquid.

Swab in transport media: vortex for 30 seconds the vial containing the swab in its transport media, then use the media directly as sample.

c) Feces

Mix 1 volume of feces (e.g., 500 µL) with an equal volume of PBS buffer. Mix vigorously by vortexing for 1 min. Allow the particles to settle down or centrifuge with low speed (e.g., at 500 x g). Proceed with the cleared supernatant. For difficult to lyse bacteria mechanical disruption or treatment using suitable beads may be required.

DETAILED PROTOCOL

This protocol is designed for magnetic separators with static pins and suitable plate shakers. It is recommended using a Square-well Block for separation or reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

1) Lyse sample

Pre-dispense **20 µL Proteinase K** and **200 µL of sample** to a suitable reaction tube. Add **180 µL Lysis Buffer NPL1 (cap with purple label)** to the reaction tube.

Optional: Add 4 µL of the Carrier RNA stock solution to the reaction tube. Mix well by repeated pipetting up and down and incubate at room temperature for 15 min with shaking.

Following the lysis incubation, spin down to collect any sample from the lysis tube lid and transfer each lysate to the wells of a Square-well Block.

Lysis incubation can be performed at 56 °C to increase the lysis efficiency e.g. for isolation of bacterial DNA from difficult to lyse bacteria.

Optionally, lysis can be supported by a pretreatment of the sample with suitable beads for mechanical disruption of difficult to lyse bacteria.

2) Bind nucleic acids to magnetic beads

Add **20 µL resuspended Magnetic Beads (vial with blue label)** and **600 µL Binding Buffer NPB2** to the lysed sample. Mix by pipetting up and down **6 times** and **shake for 5 min** at room temperature.

Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.

Magnetic Beads and Binding Buffer NPB2 can be premixed.

Be sure to resuspend the Magnetic Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has been formed.

Separate the magnetic beads by placing the vials or the plate in the magnetic separator. Wait at least 2 min until all beads have been attracted to the magnets. Remove and discard supernatant by pipetting. Do not disturb the attracted beads while aspirating the supernatant.

3) Wash with NPW3

Remove the vials or the plate from the magnetic separator.

Add **600 µL Wash Buffer NPW3** and resuspend the beads by shaking until the beads are resuspended completely (1–3 min).

Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the vials or the plate in the magnetic separator. Wait at least 2 min until all beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

4) Wash with NPW4

Remove the vials or the plate from the magnetic separator.

Add **600 µL Wash Buffer NPW4 (cap with orange label)** and resuspend the beads by shaking until the beads are resuspended completely (1–3 min).

Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the vials or the plate in the magnetic separator. Wait at least 2 min until all beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

5) Wash with 80 % ethanol

Remove the vials or the plate from the magnetic separator.

Add **600 µL 80 % ethanol** and resuspend the beads by shaking until the beads are resuspended completely (1–3 min).

Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the vials or the plate in the magnetic separator. Wait at least 2 min until all beads have

been attracted to the magnet. Remove and discard supernatant by pipetting.

6) Air dry magnetic beads
Air dry the magnetic bead pellet for 10 min at room temperature.

7) Elute RNA and DNA
Remove the vials or the plate from the magnetic separator.
Add desired volume of **Elution Buffer NPE5 (50–100 µL)** to each well and resuspend the beads by shaking 5 min at room temperature.
Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for 5 min at 56 °C.
Separate the magnetic beads by placing the vials or the plate in the magnetic separator. Wait at least 2 min until all beads have been attracted to the magnets. Transfer the supernatant containing the purified nucleic acids to either microtubes or tube strips.

TROUBLESHOOTING

1) Poor yield / low sensitivity

Incomplete sample lysis
Sample mixed with Lysis Buffer and Proteinase K was not thoroughly homogenized and mixed with Lysis Buffer and Proteinase K. The mixture has to be shaken continuously. Alternatively, prolong incubation time with Proteinase K.

Insufficient elution buffer volume
Bead pellet must be covered completely with elution buffer and needs to be fully resuspended.

Insufficient performance of elution buffer during elution step
Remove all buffer completely from the bead pellet after the binding and wash steps. Remaining buffer decreases the efficiency of the subsequent steps.

Aspiration of attracted bead pellet
Do not disturb the attracted beads while aspirating the supernatant. This requires special caution when removing the lysate from the beads as the lysate is usually too opaque to allow visual control of the pellet.

Aspiration and loss of beads
Time for magnetic separation too short or aspiration speed too high.

2) Low purity / low sensitivity

Insufficient washing procedure
Use an appropriate combinations of separator and plate. Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down.

3) Poor performance of DNA/RNA in downstream applications

Carry-over of ethanol from wash buffers
Be sure to remove all of the 80 % ethanolic wash solution from the final wash, as residual ethanol interferes with downstream applications.

Ethanol evaporation from wash buffers
Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs.

4) Carry-over of beads

Time for magnetic separation too short
Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well.

Aspiration speed too high (elution step)
High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.

WASTE MANAGEMENT

- The reagents of the kit are classified as dangerous according to Regulation EC 1272/2008 (CLP). Adopt good working practices, so that the product is not released into the environment. Recover if possible. In so doing, comply with the local and national regulations currently in force.
- Manage and waste all the biological samples as potentially infectious. All the material that come in contact with the biological sample must be treated with 0.5% sodium hypochlorite for at least 30 minutes or sterilized in autoclave at 121 °C for 30 minutes and then wasted.

BIBLIOGRAPHY

1) US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. Bloodborne Pathogens. https://www.osha.gov/lawsregs/regulations/standardnumber/1910/1910.1030

2) US Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories, 5th Ed. Washington,DC: US Government Printing Office, December 2009.

3) World Health Organization. Laboratory Biosafety Manual, 3rd ed.Geneva: World Health Organization, 2004.

4) CLSI. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline — Fourth Edition (M29-A4). Clinical and Laboratory Standards Institute, 2014.

Explanation of symbols EN

REAGENT
The terms refers to the single reagent

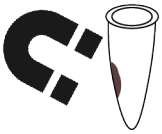
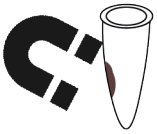
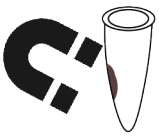
IVD *In vitro* Diagnostic Medical Device	REF Catalogue number	LOT Batch code
Cont. Contents of kit	Distributed by Distributed by	Manufacturer
Caution, consult accompanying documents Consult instructions for use		Temperature limitation
Date of Manufacture	Made in Italy Made in Italy	Use by
	Σ xxx Contains sufficient for <n> tests	Dispose of properly

Protocol at a glance

Before starting the preparation:

Check if Proteinase K and Carrier RNA were prepared according to section “Storage condition and preparation of working solution”

1	Lyse sample	20 µL Proteinase K 4 µL Carrier RNA 200 µL sample 180 µL NPL1 Mix RT, 15 min or 56°C, 15 min	
2	Bind nucleic acids to Magnetic Beads	600 µL NPB2 20 µL Beads	
		Mix by shaking for 5–10 min at RT (Optional: Mix by pipetting up and down)	
		Remove supernatant after 2 min separation	
3	Wash with NPW3	Remove the vial from magnetic separator Add 600 µL NPW3	
		Resuspend: Shake 1 min at RT	
		Remove supernatant after 2 min separation	

4	Wash with NPW4	Remove the vial from magnetic separator 600 µL NPW4	
		Resuspend: Shake 1 min at RT	
		Remove supernatant after 2 min separation	
5	Wash with 80% Ethanol	Remove the vial from magnetic separator 600 µL 80% Ethanol	
		Resuspend: Shake 1 min at RT	
		Remove supernatant after 2 min separation	
6	Drying step	Air dry for 10 min at room-temperature	
7	Elute RNA and DNA	Remove the vial from magnetic separator 50-100 µL NPE5	
		Shake 5 min at RT (Optional: Mix by pipetting up and down)	
		Separate 2 min and transfer RNA and DNA into elution plate/tubes	

SENTINEL DIAGNOSTICS
SENTINEL CH. SpA - Ph. +39 02 3455141
Via Robert Koch, 2 Milano 20152 Italy
www.sentineldiagnostics.com