RapiPREP-Nucleic Acid Extraction Kit

Instructions for Use

(Issue 2.2)

Due to the unique combination of lysis, capture and harsh washing conditions, RapiPREP-Nucleic Acid Extraction Kit enables the extraction of nucleic acids from most organisms from most sample types.

Suitable targets: Bacteria, viruses, fungi, plants, animals
Suitable samples: Blood, plasma, faeces, soil, urine, sputum
Time for extraction: 10-15 min.
Suitable for automation.

A. Intended use. For the extraction of nucleic acids from most sample types prior to testing by PCR. For *in vitro* and research use only.

B. Warning and precautions. To be used by trained personnel only. If appropriate, use within an appropriate biosafety cabinet. Wear appropriate protective clothing including gloves and laboratory coats. Dispose of all potentially infectious waste in a safe and responsible manner.

C. Kit contents (for 100 extractions):

RPNAL	10 ml	RapiPREP-NAE Lysis Solution
RPNAB	2x25 ml	RapiPREP-NAE Bead Solution
RPNAW1	80 ml	RapiPREP-NAE Wash Buffer 1
RPNAW2	80 ml	RapiPREP-NAE Wash Buffer 2
RPNAEB	10 ml	RapiPREP-NAE Elution Buffer

Do not use beyond stated expiration date. Store all reagents at room temperature.

D. Accessory reagents

A magnetic rack that holds 1.5-2.0 ml microfuge tubes to enable capture the magnetic beads.
A heating block at 80ºC that can hold 1.5-2.0 ml microfuge tubes.

E. Protocol

Preparation of material

For samples that are solid (soil, plants) or semi-solid (faeces) make a 10% (w/v) suspension in water. Solid materials such as plant material will then need blending in a blender or bead beating to break up the tissue.
Once suspensions have been made of solid or semi-solid samples, larger debris should be allowed to settle for 5 min before use. After settling, take material from the top of the liquid for lysis and extraction.
Blood, plasma, urine, sputum etc can be used directly without dilution.

Lysis of the sample

Depending on the target tissue or organism, mild, medium or harsh lysis might be required.

Mild lysis relies on lysis by the guanidinium thiocyanate in the RapiPREP-NAE Bead Solution e.g. mammalian cells, some viruses and some bacteria. This is the preferred method of choice but for hard to lyse organisms, physical bead beating may be used or the chemical lysis methods described below can be used.

Medium lysis relies on lysis by the alkali in the RapiPREP-NAE Lysis solution **PLUS** guanidinium thiocyanate in the RapiPREP-NAE Bead Solution e.g. some viruses, some bacteria, some fungi.

Harsh lysis relies on lysis by the alkali in the RapiPREP-NAE Lysis solution **PLUS** heat **PLUS** guanidinium thiocyanate in the RapiPREP-NAE Bead Solution e.g. some viruses, some bacteria (eg. Mycobacteria), some fungi, and spores.

Mild lysis for samples of blood, plasma and urine

1. Place 25 µl of sample or 10% suspension (see Preparation of material, above) in a 1.5 or 2 ml microfuge tube. Proceed to the next section – Nucleic acid capture and elution.

Medium lysis for samples of plasma, urine.

1. Place 25 µl of RapiPREP-NAE Lysis solution (RPNAL) in a 1.5 or 2 ml microfuge tube.
2. Add 25 µl of sample or 10% suspension (see Preparation of Sample, above). Mix and leave for 5 min. Proceed to the next section – Nucleic acid capture and elution.

Harsh lysis for samples of non-bloody sputum, plasma, urine

1. Place 25 µl of RapiPREP-NAE Lysis solution (RPNAL) in a 1.5 or 2 ml microfuge tube.
2. Add 25 µl of sample or 10% suspension (see Preparation of Sample, above). Mix and heat for 5 min at 95ºC. Proceed to the next section – Nucleic acid capture and elution.

Harsh lysis for samples of blood, faeces, soil and bloody samples of sputum or urine.

1. Place 150 µl of RapiPREP-NAE Lysis solution (RPNAL) in a 1.5 or 2 ml microfuge tube.
2. Add 50 µl of sample or 10% suspension (see Preparation of Sample, above). Mix and heat for 5 min at 95ºC.
3. Spin at 12 000 xg for 2 min in a microfuge. Avoid any pellet and take 100 µl of the supernatant to a fresh microfuge tube. Proceed to the next section – Nucleic acid capture and elution.

Nucleic acid capture and elution

1. To the samples prepared above, add 0.5 ml RapiPREP-NAE Bead Solution (RPNAB). Note: before using the RapiPREP-NAE Bead Solution ensure that the beads are resuspended in the solution.
2. Mix the beads with the sample and leave for one minute. Agitate the tubes to resuspend any settled beads and leave for a further 2 min Note: a longer time will not affect the result.
3. Place the tubes in a magnetic rack and once the beads are captured, remove the supernatant. If there is any liquid on the underside of the lid, remove this too as residual guanidinium will inhibit subsequent PCR.
4. Add 0.8 ml of RapiPREP-NAE Wash Buffer 1 (RPNAW1) and away from the magnet resuspend the beads. If the beads are clumped, pipette up and down a couple of times.
5. Place the tubes back in the magnetic rack and once the beads are captured, remove the supernatant. Note: as the liquid is being removed it can be used to rinse the underside of the tube lid to remove any residual guanidinium.
6. Add 0.8 ml of RapiPREP-NAE Wash Buffer 2 (RPNAW2) and away from the magnet resuspend the beads. If the beads are clumped, pipette up and down a couple of times.
7. Place back in the magnetic rack and once the beads are captured, remove the supernatant.
8. Add 50 – 100 µl of RapiPREP-NAE Elution Buffer (RPNAEB) and away from the magnet resuspend the beads. If the beads are clumped, pipette up and down a couple of times.
9. Heat the tubes at 95ºC for 5 min. At the end of this time resuspend the beads in the Elution Buffer and place in the magnetic rack. Avoiding the beads, take a volume for PCR (see below).

Molecular analysis

An appropriate PCR should be used for the nucleic acid of interest. Depending on the sample type, 2-6 µl of extract can be analysed in a 20 µl PCR. Other molecular methods can be used e.g. LAMP. The volume of the eluate from step 9 may have to be diluted by addition of water if more eluate is required for these alternative methods.