MD0667_IFU_EN_V2401

NZY Mag Universal RNA/DNA Isolation Kit, RUO

Catalogue number	Presentation
MD06671	100 preps

Description

The NZY Mag Universal RNA/DNA Isolation Kit, RUO, utilizes cutting-edge magnetic bead technology for swift and high-quality RNA and DNA purification from a wide range of complex and recalcitrant biological samples. The kit ensures that RNA/DNA samples are suitable in terms of purity, concentration, and nucleic acid integrity. Designed for compatibility with qPCR/RT-qPCR and various downstream applications, including LAMP, Helicase-dependent amplification (HDA) and digital PCR, this kit ensures rapid and a user-friendly extraction protocol. For scalability, the kit supports automated workflows on platforms like the KingFisher™ Flex (Thermo Fisher Scientific™), processing 96 specimens with a minimal extraction and reagent preparation time, to get diagnostic results more quickly. The optimized protocol on the KingFisher™ Flex automation platform allows the processing of 96 samples within 25 minutes.

The kit is optimized to address several sample biological matrices, including whole blood, plasma, serum, milk, urine, nasopharyngeal, cervical, and perianal (human) swabs, stool, environmental samples and environmental and animal faecal swabs. The NZY Mag Universal RNA/DNA Isolation Kit, RUO, establishes a new benchmark in nucleic acid purification, offering unmatched speed, simplicity, and dependability. Its innovative technology and straightforward design empower researchers to advance their studies.

Shipping & Storage Conditions

NZY Mag Universal RNA/DNA Isolation Kit, RUO, is shipped at room temperature. All components should be stored at 15 - 25 °C upon arrival. The product will remain stable till the expiry date if stored as specified.

Components

NZY Mag Universal RNA/DNA Isolation Kit, RUO, provides a comprehensive set of reagents for the simultaneous isolation and purification of DNA and RNA from a variety of biological matrices. NZY Mag Universal RNA/DNA Isolation kit is designed for the purification of 100 samples. Kit content is described in the table below:

COMPONENT	MD06671 100 PREPS
NZY Mag UniLysis Buffer	2 x 25 mL
NZY Mag UniBinding Buffer	2 x 19,5 mL
NZY Mag UniWash Buffer 1	2 x 27,5 mL
NZY Mag UniWash Buffer 2	2 x 27,5 mL
NZY Mag UniElution Buffer	9,9 mL
NZY Mag UniProteinase K	1,1 mL
NZY Mag UniBinding Beads	2,2 mL

Materials and Instrumentation Required but Not Provided

- Adjustable micropipettes
- Multi-channel micropipettes
- Disposable pipette tips (low retention, aerosol barrier and RNase/DNase-free)
- Vortex mixer or equivalent
- 96-well plates
- Disposable gloves
- Benchtop microcentrifuge capable of 15 000 x g

- Automated Extraction and Purification System
- Automatic platform-specific consumables
- Adhesive PCR Film or equivalent
- 1 x PBS, pH 7.4
- PK buffer
- Optional: Internal extraction control (IEC)

Guidelines before starting

Download and install the NZY_MD0667_Flex script

1. In case KingFisher™ Flex Magnetic Particle Processor is used, the appropriate script for NZY Mag Universal RNA/DNA Isolation Kit, RUO, must be installed on the instrument before first use. On the NZY Mag Universal RNA/DNA Isolation Kit, RUO, product web page (at www.nzytech.com) search by catalogue number and scroll to the Manuals section.
2. Download the latest version of the NZY_MD0667_Flex.bdz script for your instrument.

General recommendations

- Determine the number of required reactions based on the number of samples to be processed; include one Negative Control per plate.
- Before use, invert bottles of solutions and buffers to guarantee complete mixing.
- Yield and quality of purified nucleic acids depend on the sample type, sample source, transport, storage, age and the virus/bacteria titter.
- Before beginning the extraction procedure, review your assay documentation to determine if an IEC is recommended to verify the efficacy of the nucleic acid preparation.
- This kit was only validated for whole blood and derivative samples (plasma and serum) that contain EDTA, not citrate or heparin as an anticoagulant.
- Viral nucleic acids from biological sample material are usually very low concentrated and therefore almost impossible to quantify photometrically. Quantitative PCR/RT-PCR is recommended for yield determination.
- The yield and quality of isolated nucleic acids are generally well-suited for a variety of molecular diagnostic applications. It is recommended to carry out downstream applications following the specifications provided by the respective manufacturers.
- For cross-contamination prevention:
  – Cover the plate or tube strip during incubation and shaking to prevent spillage.
  – Pipette reagents and samples carefully to prevent splashing.
- To avoid nuclease contamination:
  – Wear lab gloves for protection from reagents and to safeguard nucleic acids from skin nucleases.
  – Decontaminate lab benches and pipettes before starting.
  – Use RNase/DNase-free pipette tips; discard used tips.

Recommended protocols

Before initiating the extraction procedure, carefully review the instructions for use. Please select the appropriate protocol from the table below based on the sample matrix and nucleic acids you intend to extract.

SAMPLE MATRIX	NUCLEIC ACID	RECOMMENDED PROTOCOL
Milk Plasma Serum Urine Ear punch/notch Swabs Tissue or organ Whole blood	Viral DNA/RNA Bacterial DNA Genomic DNA	**Standard**
Swabs Whole blood Tissue or organ	Bacterial DNA	**Digestion**
Environmental samples Faeces Swabs - environmental or faecal Oral fluid (e.g. sputum, saliva)	Viral DNA/RNA Bacterial DNA	**Robust**

Standard protocol

The Standard protocol is recommended for the following sample types: whole blood, plasma, serum, milk, urine, rinsed liquid from animal and human swabs (*e.g* nasopharyngeal, cervical, and perianal), tissue or organs, ear notch and ear punch (circular shape, 2- to 3-mm diameter; PBS incubation). The optimized nucleic acids extracted through this workflow vary with the sample type; refer to the "Recommended protocols" section for more details.

Prepare the processing plates:

1. Wash Plate 1, with 500 µL of NZY Mag UniWash Buffer 1 per well/sample.
2. Wash Plate 2, with 500 µL of NZY Mag UniWash Buffer 2 per well/sample.
3. Elution Plate, with 90 µL of NZY Mag UniElution Buffer per well/sample.

PLATE	PLATE TYPE	COMPONENT	VOLUME PER PREP
Wash Plate 1	Deep Well Plate	NZY Mag UniWash Buffer 1	500 µL
Wash Plate 2	Deep Well Plate	NZY Mag UniWash Buffer 2	500 µL
Elution Plate	Deep Well Plate	NZY Mag UniElution Buffer	90 µL

4. To avoid evaporation and contamination, cover the prepared processing plates with sealing foil until they are ready to be loaded into the instrument.

Combine Beads/Proteinase K Solution

1. Vortex the NZY Mag UniBinding Beads thoroughly to ensure that the beads are fully resuspended.
2. Combine the following components for the required number of samples plus 10% overage.

COMPONENT	VOLUME PER SAMPLE
NZY Mag UniBinding Beads	20 µL
NZY Mag UniProteinase K	10 µL
TOTAL	**30 µL**

Combine Lysis/Binding Solution

1. Combine the following components for the required number of samples plus 10% overage.

COMPONENT	VOLUME PER SAMPLE
NZY Mag UniLysis Buffer	350 µL
NZY Mag UniBinding Buffer	350 µL
TOTAL	**700 µL**

Optional: If required, internal extraction control (IEC) DNA or RNA should be added to the Lysis/Binding Solution.

2. Mix by inverting the tube or bottle at least 10 times.

Preparation of starting materials

1. Prepare the different starting materials following the indications described in the table below:

SAMPLE/MATRIX	SET-UP
Whole blood, plasma, serum, Milk or Urine	1. Always mix the sample well before extraction. 2. Proceed with 200 µL of sample.
Dry Swabs	1. Break off the tip of the swab and add to a 2-mL tube. 2. Add 1 mL of PBS, pH 7.4 to each sample. Squeeze the swab to the inner wall of the tube to obtain as much sample as possible. 3. Vortex for 3 minutes. 4. Proceed with 200 µL of supernatant.
Swabs in stabilization liquid	1. Always mix the sample well before extraction. 2. Proceed with 200 µL of the stabilization solution directly. (Note: some stabilization solution may interfere with the lysis reaction)
Tissue or organ	1. Add the following components to a 2-mL tube: ▪ Tissue: 20 to 30 mg ▪ PBS, pH 7.4: 1 mL 2. Disrupt (bead-beat) the samples in a Homogenizer at 6 m/s for 45 seconds. 3. Centrifuge at 1000 × g for 1 minute. 4. Proceed with 100 µL of supernatant.

Combine the mixture of Beads/Proteinase K, sample, and Lysis/Binding Solution

1. Invert the tube of Beads/Proteinase K Solution several times to resuspend the beads, then add 30 µL of this solution to the required wells in the plate or tube strip.
2. Transfer the appropriate volume of each prepared sample to a well with Beads/Proteinase K Solution.
3. Add 100-200 µL of sample to each sample well. May mix through a gentle up and down pipetting. Add the corresponding volume of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Mix the sample with Beads/Proteinase K Solution for 2 minutes at room temperature according to your mixing method: plate shaker - shake vigorously for 2 minutes; by pipetting - Pipette up and down several times, then incubate for 2 minutes at room temperature.
5. Add 700 µL of Lysis/Binding Solution to each sample-containing well or tube.
6. Immediately proceed to process samples on the instrument (next section).

Process samples on the automatic platform

1. Select the script NZY_MD0667_Flex.bdz on the instrument.
2. Start the automated extraction by setting up the processing plates according to the extraction system protocol.
3. After the run is completed, remove the Elution Plate from the instrument and cover it with an appropriate clear adhesive film.
4. Place the Elution Plate on ice for immediate use in real-time qPCR/RT-qPCR or store it as appropriate for later analysis.
5. For short-term storage of up to 24 hours, we recommend storing the purified viral RNA and DNA at -15 to -35°C. For storage longer than 24 hours, we recommend storing purified nucleic acids at < -65°C.

Digestion Protocol

The Digestion protocol is recommended for the following sample types: environmental samples, faeces, swabs (environmental or faecal) and tissue or organs. The optimized nucleic acids extracted through this workflow vary according to the sample type; refer to the "Recommended protocols" section. Please notice that this workflow is not recommended for the purification of RNA.

Prepare the processing plates:

1. Wash Plate 1, with 500 µL of NZY Mag UniWash Buffer 1 per well/sample.
2. Wash Plate 2, with 500 µL of NZY Mag UniWash Buffer 2 per well/sample.
3. Elution Plate, with 90 µL of NZY Mag UniElution Buffer per well/sample.

PLATE	PLATE TYPE	COMPONENT	VOLUME PER PREP
Wash Plate 1	Deep Well Plate	NZY Mag UniWash Buffer 1	500 µL
Wash Plate 2	Deep Well Plate	NZY Mag UniWash Buffer 2	500 µL
Elution Plate	Deep Well Plate	NZY Mag UniElution Buffer	90 µL

4. To avoid evaporation and contamination, cover the prepared processing plates with sealing foil until they are ready to be loaded into the instrument.

Combine Lysis/Binding Solution

1. Combine the following components for the required number of samples plus 10% overage.

COMPONENT	VOLUME PER SAMPLE
NZY Mag UniLysis Buffer	350 µL
NZY Mag UniBinding Buffer	350 µL
TOTAL	**700 µL**

Optional: Internal extraction control (IEC) DNA or RNA should be added to the Lysis/Binding Solution.

2. Mix by inverting the tube or bottle at least 10 times.

Prepare Proteinase K Solution

Prepare Proteinase K Solution immediately before use.

1. Combine the following components for the required number of samples plus 10% overage.

COMPONENT	VOLUME PER SAMPLE
PK Buffer *	90 µL
NZY Mag UniProteinase K	10 µL

** Note: PK buffer suggestions: 0.5 M EDTA pH 8, 20% SDS or 1x PBS pH 7.4.*

2. Invert the tube several times to mix, then centrifuge briefly to collect contents at the bottom of the tube.
3. Immediately proceed to the next step:
   For tube processing—proceed to "Treat the samples with Proteinase K Solution (see tube processing below).
   For plate processing—proceed to "Treat the samples with Proteinase K Solution (see plate processing below).

Treat the samples with Proteinase K Solution (tube processing)

For tube processing treat the samples with Proteinase K Solution according to the sample type:

SAMPLE/MATRIX	SET-UP
Environmental samples Faeces	1. Transfer 0.2–0.3 g of sample to a 2-mL tube. 2. Add 1 mL of PBS, pH 7.4, then vortex vigorously for 3 minutes. 3. Centrifuge at 100 × *g* for 1 minute. 4. Transfer 200 µL of the supernatant to a new tube. 5. Add 100 µL of Proteinase K Solution to the transferred supernatant, then vortex briefly to mix. 6. Incubate for 30 minutes at 55°C. 7. Centrifuge at 15 000 × *g* for 2 minutes. 8. Proceed with 200 µL of digested sample.
Swabs – environmental or faecal	1. Faecal samples - swirl a clinical swab in a faecal sample. Environmental swabs - proceed with an environmental swab.

	2. Add 1 mL of PBS, pH 7.4 to a 2-mL tube. 3. Squeeze the swab to the inner wall of the tube, for 5-10 seconds, to obtain as much sample as possible. Then discard the swab. Alternatively, break off the swab tip and leave the swab in the PBS, pH 7.4. 4. Vortex vigorously for 3 minutes, or until the sample is suspended. 5. Centrifuge at 100 × *g* for 1 minute. 6. Transfer 200 µL of the supernatant to a new tube. 7. Add 100 µL of Proteinase K Solution to the transferred supernatant, then vortex briefly to mix. 8. Incubate for 30 minutes at 55°C. 9. Centrifuge at 15 000 × *g* for 2 minutes. 10. Proceed with 200 µL of digested sample.
Tissue or organ	1. Transfer 20‑30 mg of the sample to a 2-mL tube. 2. Add 100 µL of Proteinase K Solution to the sample. 3. Incubate for 2 hours at 55 ºC. 4. Centrifuge briefly to collect the contents to the bottom of the tube. 5. Proceed with the volume of digested sample that is available to pipet. The available volume will be less than 100 µL. 6. Use a P1000 pipette tip to transfer the viscous sample.

Treat the samples with Proteinase K Solution (plate processing)

For plate processing treat the samples with Proteinase K Solution according to the sample type:

SAMPLE/MATRIX	SET-UP
Environmental samples Faeces	1. Transfer 0.2‑0.3 g of the sample to a 2-mL tube. 2. Add 1 mL of PBS, pH 7.4, then vortex vigorously for 3 minutes. 3. Centrifuge at 100 × g for 1 minute. 4. Transfer 200 µL of the supernatant to a deep-well plate. 5. Add 100 µL of Proteinase K Solution to the transferred supernatant, then pipet up and down to mix. 6. Seal the plate with sealing foil. 7. Incubate for 30 minutes at 55°C. 8. Centrifuge at 3 000 × *g* for 2 minutes. 9. Proceed with 200 µL of digested sample.
Swabs – environmental or faecal	1. Faecal samples - swirl a clinical swab in a faecal sample. Environmental swabs - proceed with an environmental swab. 2. Add 1 mL of PBS, pH 7.4 to a 2 mL tube. 3. Squeeze the swab to the inner wall of the tube, for 5-10 seconds, to obtain as much sample as possible. Then discard the swab. Alternatively, break off the swab tip and leave the swab in the PBS, pH 7.4. 4. Vortex vigorously for 3 minutes, or until the sample is suspended. 5. Centrifuge at 100 × g for 1 minute. 6. Transfer 200 µL of the supernatant to a deep-well plate. 7. Add 100 µL of Proteinase K Solution to the transferred supernatant, then pipet up and down to mix. 8. Seal the plate with sealing foil. 9. Incubate for 30 minutes at 55°C. 10. Centrifuge at 3 000 × *g* for 2 minutes. 10. Proceed with 200 µL of digested sample.
Tissue or organ	1. Transfer 20 ‑30 mg of the sample to a 2-mL tube. 2. Add 100 µL of Proteinase K Solution to each sample. 3. Seal the plate with sealing foil. 4. Incubate for 2 hours at 55 ºC. 5. Centrifuge briefly to collect the contents to the bottom of the tube. 6. Proceed with the volume of digested sample that is available to pipet. The available volume will be less than 100 µL. Use a P1000 pipette tip to transfer the viscous sample.

Prepare the mixture of Samples treated with Proteinase K Solution and Lysis/Binding Solution

1. Vortex the NZY Mag UniBinding Beads several times to resuspend the beads, then add 20 µL of the beads to the required wells in the plate or tube.
   Note: Do not use Beads/Proteinase K Solution.
2. Add the appropriate volume of each Proteinase K-treated sample to a well with beads.

SAMPLE/MATRIX	VOLUME PER SAMPLE
Environmental samples, faeces swabs	200 µL
Tissue or organs	Up to 100 µL

3. Mix the sample with beads for 2 minutes at room temperature according to your mixing method: plate shaker - shake vigorously for 2 minutes; by pipetting - Pipette up and down several times, then incubate for 2 minutes at room temperature.
4. Add 700 µL of Lysis/Binding Solution to each sample-containing well or tube.
5. Immediately proceed to process samples on the instrument (next section).

Process samples on the automatic platform

1. Select the script NZY_MD0667_Flex.bdz on the instrument.
2. Start the automated extraction by setting up the processing plates according to the extraction system protocol.
3. After the run is completed, remove the Elution Plate from instrument and cover it with an appropriate clear adhesive film.
4. Place the Elution Plate on ice for immediate use in real-time PCR or store it as appropriate for later analysis.
5. For short-term storage of up to 24 hours, we recommend storing the purified nucleic acids at -15 to -35°C. For storage longer than 24 hours, we recommend storing purified nucleic acids at < -65°C.

Robust Protocol

The Robust protocol is recommended for more complex sample types when compared with the standard approach, such as saliva/sputum (oral fluid), environmental samples, faeces, and swabs (environmental or faecal). The optimized nucleic acids extracted through this workflow vary according to the sample type; refer to the "Recommended protocols" section.

Prepare the processing plates:

1. Wash Plate 1, with 500 µL of NZY Mag UniWash Buffer 1 per well/sample.
2. Wash Plate 2, with 500 µL of NZY Mag UniWash Buffer 2 per well/sample.
3. Elution Plate, with 90 µL of NZY Mag UniElution Buffer per well/sample.

PLATE	PLATE TYPE	COMPONENT	VOLUME PER PREP
Wash Plate 1	Deep Well Plate	NZY Mag UniWash Buffer 1	500 µL
Wash Plate 2	Deep Well Plate	NZY Mag UniWash Buffer 2	500 µL
Elution Plate	Deep Well Plate	NZY Mag UniElution Buffer	90 µL

4. To avoid evaporation and contamination, cover the prepared processing plates with sealing foil until they are ready to be loaded into the instrument.

Combine Beads/Proteinase K Solution

1. Vortex the NZY Mag UniBinding Beads thoroughly to ensure that the beads are fully resuspended.
2. Combine the following components for the required number of samples plus 10% overage.

COMPONENT	VOLUME PER SAMPLE
NZY Mag UniBinding Beads	20 µL
NZY Mag UniProteinase K	10 µL
TOTAL	**30 µL**

Prepare Lysis Solution (optional)

1. Combine the following components for the required number of samples plus 10% overage.

COMPONENT	VOLUME PER SAMPLE
NZY Mag UniLysis Buffer	450 µL
***Optional:* IEC**	X µL
TOTAL	**450 µL + X volume of IEC**

Optional: If required, Internal extraction control (IEC) DNA or RNA should be added to the Lysis solution.

2. Mix by inverting the tube or bottle at least 10 times.

Preparation of the clarified lysate

1. Prepare the different clarified lysates following the indications described in the table below:

SAMPLE/MATRIX	SET-UP
Environmental samples Faeces	1. Transfer 0.2-0.3 g of the sample to a 2-mL tube. 2. Add 1 mL of PBS, pH 7.4, then vortex vigorously for 3 minutes. 3. Centrifuge as indicated. For viral nucleic acid purification - centrifuge at 15 000 × g for 1 minute. For bacterial DNA purification or concurrent purification of bacterial and viral nucleic acids - centrifuge at 100 × g for 1 minute. 4. Proceed with 200 µL of supernatant.
Oral fluid	1. Briefly mix the oral fluid sample. 2. Proceed with 300 µL of sample.
Swabs - environmental or faecal	1. Faecal samples - swirl a clinical swab in a faecal sample. Environmental swabs - proceed with an environmental swab. 2. Add 1 mL of PBS, pH 7.4 to a 2-mL tube. 3. Squeeze the swab to the inner wall of the tube, for 5-10 seconds, to obtain as much sample as possible. Then discard the swab. Alternatively, break off the swab tip and leave the swab in the PBS, pH 7.4. 4. Vortex vigorously for 3 minutes, or until the sample is suspended.

	5. Centrifuge as indicated: For viral nucleic acid purification–centrifuge at 15 000 × g for 1 minute. For bacterial DNA purification or concurrent purification of bacterial and viral nucleic acids Centrifuge at 100 × g for 1 minute. 6. Proceed with 200 µL of supernatant.

2. Add NZY Mag UniLysis Buffer (or Lysis Solution, when adding IEC) and then clarify the lysate:

PROCESSING IN	SET-UP
Tubes	1. For each sample, add 450 µL of NZY Mag UniLysis Buffer to a new 2-mL tube. 2. Add the indicated volume of sample from the previous step to the NZY Mag UniLysis Buffer. 3. Vortex vigorously for 3 minutes. 4. Centrifuge at 15 000 × g for 2 minutes. 5. Remove the supernatant (clarified lysate) without disturbing the pellet.
Plates	1. For each sample, add 450 µL of NZY Mag UniLysis Buffer to the appropriate wells of a deep-well plate. 2. Add the indicated volume of sample from the previous step to NZY Mag UniLysis Buffer. 3. Seal the plate with sealing foil. 4. Shake the plate at moderate speed for 5 minutes. 5. Centrifuge at 3 000 × g for 5 minutes. 6. Remove the supernatant (clarified lysate) without disturbing the pellet.

Prepare the clarified Lysate with Beads/Proteinase K Solution and NZY Mag UniBinding Buffer

1. Invert the tube of Beads/Proteinase K Solution several times to resuspend the beads, then add 30 µL of this solution to the required wells in the plate or tube strip.
2. Transfer the appropriate volume of each clarified lysate to a well with Beads/Proteinase K Solution.
3. Add 600 µL of oral fluid clarified lysate or 500 µL of environmental samples, faecal samples, and swabs clarified lysate, to each sample well. May mix through a gentle up and down pipetting. Add the corresponding volume of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Mix the sample with Beads/Proteinase K Solution for 2 minutes at room temperature according to your mixing method: plate shaker - shake vigorously for 2 minutes; by pipetting - Pipette up and down several times, then incubate for 2 minutes at room temperature.
5. Add 350 µL of Mag UniBinding Buffer.
6. Immediately proceed to process samples on the instrument (next section).

Process samples on the automatic platform

1. Select the script NZY_MD0667_Flex.bdz on the instrument.
2. Start the automated extraction by setting up the processing plates according to the extraction system protocol.
3. After the run is completed, remove the Elution Plate from instrument and cover it with an appropriate clear adhesive film.
4. Place the Elution Plate on ice for immediate use in real-time PCR/RT-qPCR or store it as appropriate for later analysis.
5. For short-term storage of up to 24 hours, we recommend storing the purified viral RNA and DNA at -15 to -35°C. For storage longer than 24 hours, we recommend storing purified nucleic acids at < -65°C.

Data

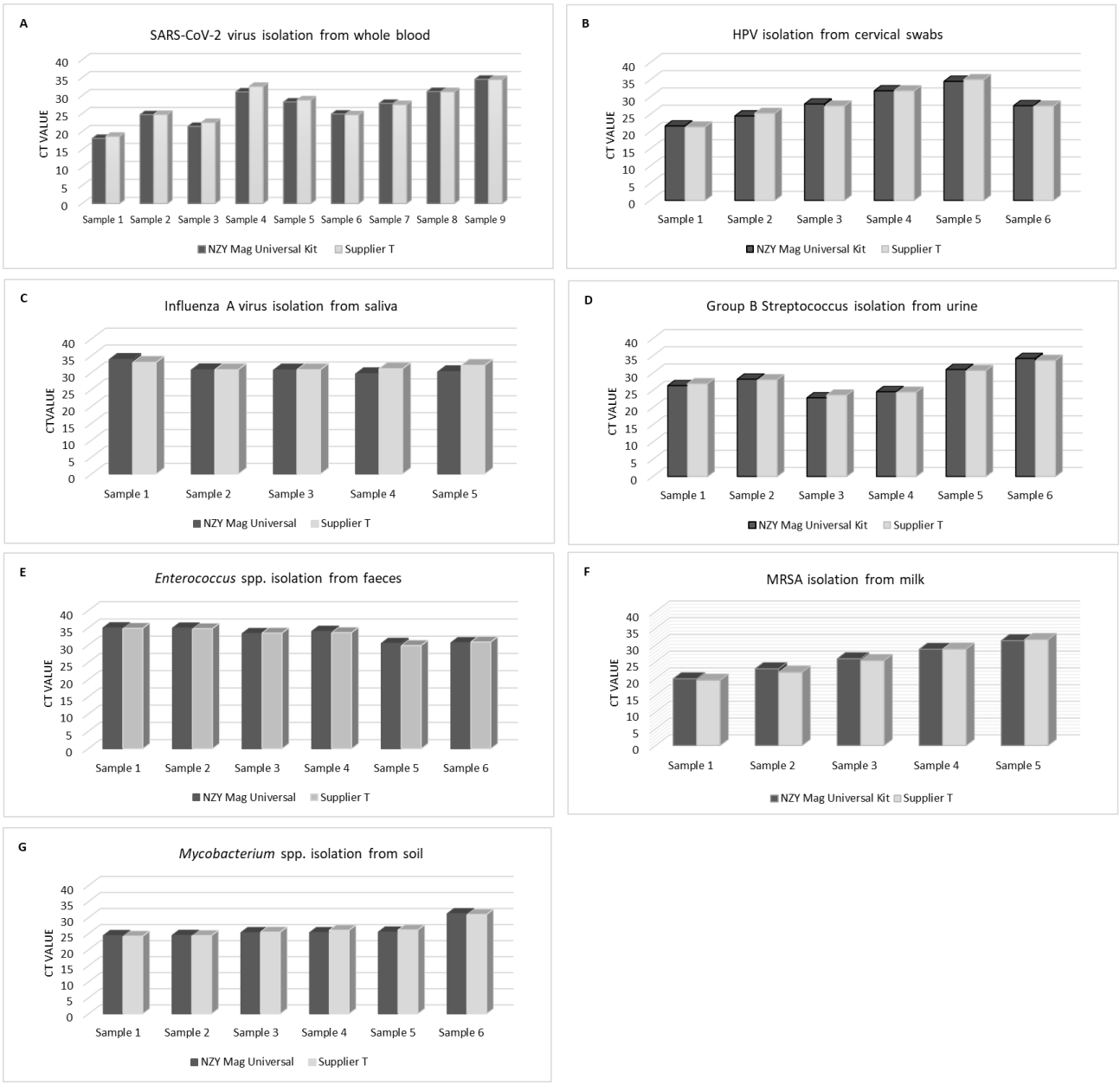

Figure 1. Comparative performance of the NZY Mag Universal RNA/DNA Isolation Kit, RUO, and an alternative commercially available kit (Supplier T) in Extracting Viral and Bacterial Pathogens Across Various Sample Matrices: A. SARS-CoV-2 virus isolation from whole blood; **B.** Human papillomavirus (HPV) isolation from cervical swabs; **C.** Influenza A virus isolation from saliva; **D.** Group B Streptococcus isolation from urine; **E.** *Enterococcus spp.* isolation from faeces; **F.** Methicillin-resistant *Staphylococcus aureus* (MRSA) *mec*A gene isolation from milk; **G.** *Mycobacterium spp.* isolation from soil.

Quality control

All components of NZY Mag Universal RNA/DNA Isolation Kit, RUO, are tested following the protocols described above by the extraction of nucleic acids from bacterial DNA and viral RNA from different matrices. The inclusion of negative and positive samples in addition to no-template controls is used to test extraction efficacy and cross-contamination with non-targeted nucleic acids.