NZY Mag Viral RNA/DNA Isolation Kit, IVD

REF MD04881, 200 preps
MD04882, 2000 preps

For professional* in-vitro *diagnostic use only

CE IVD

EN

Instructions for Use (IFU)

IM-005en

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NZYTech genes & enzymes
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Contents

1. Intended Use

NZY Mag Viral RNA/DNA Isolation Kit, IVD, is a magnetic bead technology-based nucleic acid purification kit intended for the rapid and simple purification of viral RNA and DNA of the highest integrity from human nasopharyngeal swabs. The kit is designed to be used with a variety of downstream applications requiring amplification and detection of viral RNA/DNA, in particular RT-qPCR. NZY Mag Viral RNA/DNA Isolation kit, IVD, is intended for use by professional users experienced and trained in molecular biological techniques, including experience with swabs and potentially infectious human sample materials.

2. Principles of the Assay

NZY Mag Viral RNA/DNA Isolation Kit, IVD, is specifically designed to recover RNA and DNA from viral particles contained in transport medium from human respiratory swabs. The kit uses magnetic-bead technology to provide reproducible recovery of high-quality nucleic acids. The procedure is based on the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. A homogeneous distribution of the magnetic beads is essential for a high isolation consistency. Therefore, before dispensing NZY Mag Binding Beads make sure that beads are completely resuspended. Sample lysis and binding to the NZY Mag Binding Beads is achieved by incubation with NZY Mag Binding Buffer containing chaotropic ions supported by nucleic acid release with NZY Mag Proteinase K digestion. Following nucleic acid magnetic separation, paramagnetic beads holding the RNA/DNA are washed to remove contaminants and salts using NZY Mag Wash Buffer and 80% ethanol. Residual ethanol from previous wash steps is removed by air-drying. Finally, highly pure viral RNA/DNA is eluted with NZY Mag Elution Buffer. It is highly recommended to use extraction controls, positive and negative controls, internal controls as well as to perform cross-contamination assays during the usage of NZY Mag Viral RNA/DNA Isolation kit, IVD.

Protocol options for NZY Mag Viral RNA/DNA Isolation Kit, IVD, support automated workflows in a variety of platforms, including the KingFisher™ Flex Magnetic Particle Processor with a 96 deep-well head. Automated workflows allow for 96 nasopharyngeal swab specimens to be processed in <30 minutes. In addition, a flexible protocol accommodates sample volume inputs of 200 µL of transport medium with an elution volume of 50 µL. High-quality nucleic acids are subsequently ready to use for detection using different amplification strategies such as Reverse Transcription (RT)-PCR or RT-qPCR. NZY Mag Viral RNA/DNA Isolation kit, IVD, does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay depending on the target pathogen. Any diagnostic results generated using nucleic acids isolated with the NZY Mag Viral RNA/DNA Isolation Kit, IVD, in conjunction with an *in vitro* diagnostic assay should be interpreted considering additional clinical or laboratory findings.

3. Kit Contents

NZYTech NZY Mag Viral RNA/DNA Isolation Kit, IVD, provides a comprehensive set of reagents for the isolation and purification of viral RNA and DNA from human nasopharyngeal swabs. NZY Mag Viral RNA/DNA Isolation kits are designed for the purification of 200 (#MB48801) or 2000 (#MB48802) samples, respectively, with a 200 µL sample volume input. Kit contents for the two kit presentations are as described in the Table below.

Kit Component	MD04881 (200 preps)	MD04882 (2000 preps)
NZY Mag Binding Buffer	53 mL	530 mL
NZY Mag Proteinase K	1 mL	10 mL
NZY Mag Wash Buffer	100 mL	1000 mL
NZY Mag Elution Buffer	10 mL	100 mL
NZY Mag Binding Beads	2 mL	20 mL

4. Storage, Stability and Handling Conditions

NZY Mag Viral RNA/DNA Isolation Kit, IVD, is shipped at 15 - 25 °C. All components should be stored at 15 - 25 °C upon arrival.

- If the package that protects the kit arrived damaged, please contact NZYTech.
- Beware to the expiry date indicated on the packaging. NZYTech does not recommend using the kit after the expiry date. On this date, the kit must be discarded following the disposal instructions in **Section 7.2**.

5. Materials and Instrumentation Required but Not Provided

The instrumentation required may vary depending on processing and setup or configuration. Please check with your local platform manufacturer regarding platform-specific consumables. Use disposable pipet tips (aerosol barrier and RNase-free pipet tips are recommended to avoid cross-contaminations). Use personal protection equipment including disposable gloves.

6. Sample Collection and Preparation

Different factors, such as protocol for sample collection from human respiratory specimen (nasopharyngeal or oropharyngeal swabs), sample transport, storage, and processing time, are critical to achieve optimal results. The collected samples should be tested as soon as possible. Samples should be transported and stored at low temperatures in accordance with biosafety regulations. NZYTech NZY Mag Viral RNA/DNA Isolation Kit, IVD, leads to the isolation of total nucleic acids that constitute the starting material for diagnostic testing. The kit ensures that RNA/DNA samples are suitable in terms of purity, concentration, and nucleic acid integrity. An $A_{260/280}$ ratio of ~2 is generally accepted for pure RNA/DNA. Since ethanol is a strong Real-Time PCR inhibitor, it is necessary to eliminate it prior to the elution of the nucleic acid during extraction and this occurs at the final stages of the extraction.

7. Precautions and Warnings

As in any analytical testing procedure, good laboratory practices are essential. Carefully follow the procedures and guidelines provided in this handbook to ensure that the test is performed correctly. Any deviation from them may result in assay failure or cause erroneous results.

7.1 Safety Information

Biological samples such as tissues, body fluids, pathogenic agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Before using the kit please consult the Safety Data Sheet (SDS) that is available at NZYTech website (www.nzytech.com). Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document. Isolation and detection of SARS-CoV-2 or other pathogenic organisms should be performed only by staff trained in the relevant technical and safety procedures in appropriately equipped laboratories. International and national guidelines on laboratory biosafety should be followed in all circumstances.

7.2 Handling and Procedural Requirements

- Only for professional *in vitro* diagnostic use.
- Do not use this kit after expiration date.
- Do not use the test components if kit sealing is damaged.
- Do not interchange reagents of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- DNase/RNase-free disposable plasticware and pipettes should be used in all procedures.
- Use DNase/RNase-free filter tips throughout the protocol to prevent aerosol and liquid contamination.
- Biological samples must be handled as if they are infectious following proper biosafety precautions.
- Chemical residues are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations.
- Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations.
- Sample preparation, reaction set up and amplification after subsequent testing should be performed in different working areas.
- In case of testing, handle post-amplification plates with care and dispose them immediately after the end of the assay; plates should always be discarded into a proper biohazard container after use.
- All results should be interpreted by a health-care professional in the context of patient medical history and clinical symptoms.

8. Reagent Preparation

Preparation of 80% (v/v) ethanol solution: Before start, prepare fresh 80% (v/v) ethanol [not included] by diluting absolute ethanol molecular biology grade in nuclease-free water (not DEPC-Treated) at a volume required for the number of purification reactions plus 10% overage.

Reagent	Sample volume input, 200 µL		
	Volume per prep	Volume for 10 preps (+10%)	Volume for 96 preps (+10%)
80% (v/v) ethanol	500 µL	5,50 mL	52,8 mL

9. Nucleic Acid isolation

Please read the instructions for use carefully before you begin the extraction procedure. Review your assay documentation to determine if an extraction control is recommended to verify the efficacy of the nucleic acid preparation. Follow the extraction control guidelines provided in the assay documentation. Determine the number of required reactions based on the number of patient samples to be processed, plus one Negative Control per plate.

10. Protocol for Automated Extraction

10.1 Preparation of Processing Plates

In the automated platform prepare the following processing plates:

1. **Wash Plate 1**, with 0,5 mL of NZY Mag Wash Buffer per well/sample.
2. **Wash Plate 2**, with 0,5 mL of 80% (v/v) ethanol [not provided; see guidelines to prepare above] per well/sample.
3. **Elution Plate**, with 50 µL of NZY Mag Elution Buffer per well/sample.

Plate	Plate type	Component	Volume per prep
Wash Plate 1	Deep Well Plate	NZY Mag Wash Buffer	0,5 mL
Wash Plate 2	Deep Well Plate	80% (v/v) ethanol	0,5 mL
Elution Plate	Deep Well Plate	NZY Mag Elution Buffer	50 µL

10.2 Processing Samples

1. In the **Sample Plate**, carefully pipette 265 µL of NZY Mag Binding Buffer to each sample well, as well as to the Negative Control well.

   Note: *NZY Mag Binding Buffer is intrinsically viscous and should be pipetted gently using low retention tips.*

2. Accordingly, add 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.

3. Add 5 µL of NZY Mag Proteinase K to each well, including the Negative Control well.

   Note: *At this stage may add Extraction Control if this is required*.

4. Mix well by repeated pipetting up and down or shaking.
5. Incubate reaction for at least 15 min at room temperature (***may prepare processing plates as described above during this period***).
6. Vortex NZY Mag Binding Beads to ensure that the bead mixture is homogeneous. Add 10 µL of NZY Mag Binding Beads per well/sample.
7. In case KingFisher™ Flex Magnetic Particle Processor is used, ensure that the NZY_2Wash_200_Flex program was loaded onto the instrument.
8. Start the automated extraction by setting up the processing plates according to the extraction system protocol.
9. After run is completed, remove the Elution Plate from instrument and cover with an appropriate clear adhesive film.
10. Place the Elution Plate on ice for immediate use in real-time RT-qPCR or store as appropriate for later analysis.
11. For short-term storage of up to 24 hours, we recommend storing the purified viral RNA and DNA at 2–8°C. For storage longer than 24 hours, we recommend storing purified nucleic acids at < -70°C.

11. Performance Evaluation

Evaluation of NZYTech NZY Mag Viral RNA/DNA Isolation Kit, IVD, performance was carried out using an automated workflow in a KingFisher™ Flex Magnetic Particle Processor with a 96 deep-well head with an elution volume of 50 µL. Performance studies described here were performed by extracting nucleic acids from cytomegalovirus (CMV), a DNA herpesvirus, and SARS-CoV-2, a virus with an RNA genome. Nucleic acid isolation efficacy was tested through the quantification of isolated nucleic acids by real-time qPCR using 8 µL of each nucleic acid extract with CMV (#MD0341) and SARS-CoV-2-specific (SARS-CoV-2 One-Step RT-qPCR kit, cat. nº MD0483) NZYTech testing kits using an Applied Biosystems™ 7500 FAST platform.

11.1 Limit of Detection (LoD) - Analytical Sensitivity

The analytical sensitivity was defined as the lowest viral load in a clinical sample from which nucleic acids could be reliably isolated with a 95% confidence. This was assessed by testing the extraction of nucleic acids from different copy numbers of CMV and SARS-CoV-2 virus spiked into a pool of negative human nasopharyngeal swabs, using 3 different kit batches and following typical isolation conditions. Tests were repeated over 2 days, producing 24 replicates for each CMV or SARS-CoV-2 viral load. Together, the data revealed that NZYTech NZY Mag Viral RNA/DNA Isolation Kit, IVD, can efficiently isolate nucleic acids from clinical samples containing 50 copies of CMV or SARS-CoV-2 virus per reaction with a confidence ≥95%. Thus, the kit Limit of Detection (LoD) was determined to be 0.25 viral copies/µL in the input sample.

11.2 Clinical Equivalence and Cross-Isolation

Clinical equivalence was tested through the isolation of nucleic acids from CMV and SARS-CoV-2 using NZYTech NZY Mag Viral RNA/DNA Isolation Kit, IVD, and a commercial IVD kit employing the same extraction protocol (Applied Biosystems™, MagMAX™ Viral/Pathogen II Nucleic Acid

Isolation Kit, CE-IVD, #A48383). Tests were performed using a pool of negative human nasopharyngeal swabs contrived with the two viruses at four different copy numbers (1000, 500, 100 and 50 viral copies/200 µL reaction). In total, nucleic acids were isolated from 80 samples with the two kits (160 extractions per virus). The tests were implemented with CMV and SARS-CoV-2 virus. The data revealed no significant differences in extraction efficacy when the two kits were compared, as presented in the Table below.

Clinical equivalence of NZY Mag Viral RNA/DNA Isolation Kit				
SARS-CoV-2 copies/reaction	**Ct Mean**	**SD**	**CV (%)**	**n**
1000	29,71	0,30	1,02	40
500	31,06	0,26	0,83	40
100	33,56	0,32	0,96	40
50	34,74	0,80	2,32	40
CMV copies/reaction	**Ct Mean**	**SD**	**CV (%)**	**n**
1000	29,60	0,50	1,68	40
500	30,95	0,64	2,06	40
100	33,61	0,67	2,01	40
50	34,71	0,81	2,34	40

The capacity of NZYTech NZY Mag Viral RNA/DNA Isolation Kit, IVD, to co-isolate RNA/DNA of endogenous origin (human), when isolating viral nucleic acids from human samples was also evaluated. The analysis of the co-purification capacity of NZYTech kit was evaluated in comparison with the Applied Biosystems™ kit equivalent (#A48383). Thus, the above samples were tested for the presence of the human RP target in the context of SARS-CoV-2 detection. The data revealed that both kits displayed similar capacity to isolate nucleic acids of human origin (average Cts of 24,54 with a Standard Deviation of 0,22 and a Coefficient of Variation of 0,90 %, n=160) during the isolation of viral RNA/DNA. Detection of the human RP gene is used to validate RT-qPCR test efficacy when using human nasopharyngeal swabs for molecular diagnostics.

11.3 Interfering Substances

The impact of 17 potential interferent substances in nucleic acids isolation efficacy was assessed in extractions of human negative nasopharyngeal specimens contrived with SARS-CoV-2 positive specimens. Potential interfering substances were added to the contrived samples at concentrations representing the highest levels expected in human respiratory patient samples based on literature data. All nucleic acids extractions were performed in pentaplicate. Efficacy of nucleic acids extraction was developed as described above by detecting/quantifying the presence of viral RNA through RT-qPCR. Data was compared with samples subjected to the same protocol but not exposed to the interferent substances. At the concentrations tested, the results revealed that none of the molecules under test affected the sensitivity of the extraction, quantified through RT-qPCR. The table below shows the data collected under these experiments. All experiments were run on the 7500 FAST Real-time PCR Instrument (Applied Biosystems™).

Potential Interferent	Active Ingredient	Final concentration in sample	Interference Yes (Y) or No (N)
Isotonic Sea Water (Rhinomer)	NaCl	15% v/v	N
Throat spray, oral anesthetic and analgesic (Strepfen)	Flurbiprofen	5% v/v	N
Nasal wash solution (Allergy spray - Vibrocil)	Fluticasone propionate	5% v/v	N
Nasal Corticosteroids spray (Nasomet)	Mometasone furoate	5% v/v	N
Nasal Corticosteroids spray (Pulmicort)	Budesonide	5% v/v	N
Antimicrobial, systemic (Trobex)	Trobamycin	600 µg/mL	N
Mouth analgeic, anti-inflamatory and antiseptic (Pyralvex)	Rhubard extract, Salicylic acid	5% v/v	N
Antifungal and Antibacterial Oropharyngeal Topic (Daktarin)	Miconazole	5 mg/mL	N
Mouthwash solution antiseptics (Eludril Gé)	Chlorhexidine gluconate, Chlorobutanol hemihydrate	5% v/v	N
Antitussive, Syrup (Codipront)	Codeine, Phenyltoloxamine citrate	5% v/v	N
Whole Blood (human)	-	4% v/v	N
Antiviral drug (Tamiflu)	Oseltamivir	7,5 mg/mL	N
Mucolytic (Mucolsovan)	Ambroxol hydrochloride	5% v/v	N
Nasal drops solution (Nasarox)	Oxymetazoline Chlorhydrate	10 % v/v	N
Antibiotic, nasal ointment (Bactroban)	Mupirocin	5 mg/mL	N
Saliva (human)	-	25% v/v	N
Absolute ethanol	Alcohol	5% v/v	N

11.4 Precision

Assay precision for the NZYTech NZY Mag Viral RNA/DNA Isolation Kit, IVD, was determined by testing the extraction of SARS-CoV-2 nucleic acids from 24 different positive human nasopharyngeal swabs, with variable viral loads, using 3 different kit batches, following typical extraction conditions with elution in 50 µL. Precision was evaluated by measuring Cq average, Cq coefficient of variation and % of replicate detection, as described below for each case and using NZYTech´ SARS-CoV-2 One-Step RT-qPCR kit, IVD (NZYTech MD0483) to quantify viral nucleic acids. The data is shown in the Table displayed below.

11.4.1. Repeatability

Repeatability was assessed by one operator by extracting 24 different samples using three different kit batches, accounting for a final number of 72 nucleic acid isolations performed.

11.4.2. Operator Reproducibility

Operator reproducibility was assessed through the extraction of 3 replicates of 24 samples, using three different kit batches, by two different operators, with 72 sample extractions performed per operator with a total of 144 samples extracted.

11.4.3. Daily Reproducibility

Daily reproducibility was assessed by one operator through the extraction of 3 replicates of 24 samples, using three different kit batches, in two different days, accounting for a final number of 144 nucleic acid isolations performed.

11.4.4. Lot-to-lot Reproducibility

Reproducibility between lots was assessed through the implementation of 216 extractions using 3 different kit batches with a total of 72 extractions per batch.

Precision of NZYTech NZY Mag Viral RNA/DNA Isolation Kit, IVD. Nucleic acids were extracted from SARS-CoV-2 positive human nasopharyngeal swabs and isolated RNA quantified through RT-qPCR using a SARS-CoV-2 One-Step RT-qPCR kit, IVD (NZYTech MD0483) that uses the human RP gene as the internal control.

Variable tested		SARS-CoV-2	RP
Repeatability			
	n	72	72
	Mean Cq	30,90	24,65
	Coefficient of Variation (%)	3,38	1,42
	% Replicate Detection	100	100
Operator Reproducibility	n	144	144
	Mean Cq	30,86	24,28
	Coefficient of Variation (%)	3,31	1,92
	% Replicate Detection	99	99
Daily Reproducibility	n	144	144
	Mean Cq	30,86	24,39
	Coefficient of Variation (%)	3,28	1,63
	% Replicate Detection	99	99
Lot-to-lot Reproducibility	n	216	216
	Mean Cq	30,85	24,23
	Coefficient of Variation (%)	3,26	1,70
	% Replicate Detection	99	99

11.5 Clinical evaluation

The clinical performance of NZYTech NZY Mag Viral RNA/DNA Isolation Kit, IVD, was evaluated by an external laboratory through the extraction of nucleic acids from human nasopharyngeal swab samples infected with virus. In total, 62 clinical positive samples were tested in parallel with a second IVD kit. Data revealed that NZY Mag Viral RNA/DNA Isolation Kit (MD0488) displayed identical sensibility when compared with the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (#A48383; Applied Biosystems™).

12. Quality Control

All components of NZYTech NZY Mag Viral RNA/DNA Isolation Kit, IVD, are tested following the protocols described above by the extraction of nucleic acids from DNA and RNA virus. 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.

13. Technical Support

For Technical support, please contact our dedicated technical support team by Phone: +351 (0) 21 364 35 14 or Email: info@nzytech.com.

14. Trademarks and Disclaimers

All trademarks that appear in this manual are the property of their respective owners.

15. Explanation of Symbols

IVD	*in vitro* diagnostic medical device		Consult instructions for use
REF	Catalogue number		Manufacturer
LOT	Batch code		Use by
	Temperature limitation		Sufficient for
	Safety warning*		Health hazard*

* For more information, please refer to the Safety Data Sheet and the Safety Instructions

16. References

Paysic J. et al- (2015). Standardization of Nucleic Acid Tests for Clinical Measurements of Bacteria and Viruses. J Clin Microbiol., 53(7), 2008–14.

Cohen, J. et al. (2016) Infectious Diseases, Elsevier, 4th ed., ISBN: 9780702062858.

Vemula S. V. et a. (2016) Current Approaches for Diagnosis of Influenza Virus Infections in Humans, Viruses 8(4), 96.

17. Conformity Declaration

Product Name: NZY Mag Viral RNA/DNA Isolation Kit, IVD

Catalogue Number: MD04881 and MD04882.

Intended use: Nucleic Acid Isolation from virus.

Manufacturer: NZYTech - Genes & Enzymes
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We, NZYTech, Lda – Genes & Enzymes, hereby declare that this product, to which this declaration of conformity relates, is in conformity with the following standards and other normative documents ISO 9001:2015, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices as transposed into the national laws of the Member States of the European Union.

The product technical file is maintained at NZYTech, Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal.

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