Polaris®
Next Gen_MDx

Polaris® Taq Polymerase 50 U/µL, IVD

REF MD06691, 0.35 mL (5 000 R)
MD06692, 3.5 mL (50 000 R)

For professional* in vitro *diagnostic use only

CE IVD

EN

Instructions for Use

MD0669_IM_en

VERSION 2401, May 2024

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NZYtech genes & enzymes

Introducing the Polaris® brand

NZYtech, with its established expertise in enzyme development and IVD kit production, proudly introduces Polaris® - a groundbreaking series of newly developed diagnostic enzymes, master mixes and reagents. Polaris® brand products set the standard in purity, superior stability, diagnostic performance, reliability, and regulatory compliance. These attributes are housed in functional packaging tailored for stringent laboratory applications. Polaris® stands at the forefront of innovation, designed to meet the complex demands of molecular diagnostics with a steadfast focus on quality and scientific integrity. At its core, Polaris® adheres to stringent international quality standards, including ISO 13485 and ISO 9001, ensuring its enzymes and reagents are perfectly suited for a wide array of IVD applications. These products surpass the stringent European IVDR requirements, demonstrating a commitment to quality management and excellence in every aspect of their development and production. Utilizing cutting-edge manufacturing protocols, precise control measures, and rigorous validation, Polaris® becomes the new benchmark for human diagnostic testing. NZYtech's state-of-the-art facilities are optimized to produce these high-precision diagnostics tools, ensuring unmatched accuracy and performance. Our team is always ready to offer comprehensive support to our customers and partners, assisting with IVDR compliance and ensuring smooth integration, upon request. NZYtech is committed to advancing the field of molecular diagnostics, thereby expanding access to clinical results, enabling rapid diagnostics, and fostering research advancement.

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1. Introduction

Polaris® Taq Polymerase 50 U/µL, IVD, designed for *in vitro* diagnostic (IVD) applications, is an optimized and highly robust diagnostic tool, meticulously designed to meet the high demands of modern medical diagnostics. The formulation boasts a high concentration of 50 U/µL, designed to facilitate the concentrated bulk production of PCR master mixes. Despite containing 50% glycerol to enhance stability and protect against freezing conditions, its high concentration ensures minimal glycerol incorporation into master mixes, thus maintaining compatibility with lyophilization processes. The result is a highly versatile enzyme that retains the reproducible performance expected in diagnostic applications, even when used in lower volumes. Accommodating a broad spectrum of PCR requirements, Polaris® Taq Polymerase 50 U/µL, IVD, is especially suited for challenging and multiplex PCR applications that demand rapid amplification without compromising specificity or sensitivity.

Polaris® Taq Polymerase 50 U/µL, IVD, is produced under a rigorous quality management system according to global standards for medical devices and IVD manufacturing, including stringent quality assurance, full traceability from production, and reliability in its diagnostic applications. The efficacy of Polaris® Taq Polymerase 50 U/µL, IVD, is especially relevant in the field of molecular diagnostics, as evidenced in this instructions for use through extensive validation for detecting a wide range of viral and bacterial pathogens, including SARS-CoV-2, Respiratory Syncytial Virus (RSV, subtypes A and B), Influenza (types A and B), methicillin-resistant *Staphylococcus aureus* (MRSA), and 14 high-risk types of Human Papillomavirus (HPV) in human clinical specimens. The comprehensive validation of Polaris® Taq Polymerase 50 U/µL, IVD, across diverse clinical matrices, establishes it as a validated Class A IVD reagent for molecular diagnostics.

2. Validation and Applications

Polaris® Taq Polymerase 50 U/µL, IVD, has undergone extensive validation studies to ensure its performance and compliance with Class A IVD requirements. The most important features of this product are:

- **Sensitivity and Specificity:** The Polaris® Taq Polymerase 50 U/µL, IVD, demonstrates exceptional sensitivity and specificity in the detection of target nucleic acids. It has been validated to detect low levels of genetic material, while the specificity of the enzyme ensures accurate differentiation between closely related genetic sequences, minimizing the risk of cross-reactivity and false-positive results.
- **Accuracy and Reproducibility:** Accuracy is paramount in diagnostic testing, and the Polaris® Taq Polymerase 50 U/µL, IVD, delivers results with high precision. Validation studies confirm the enzyme's ability to consistently produce accurate and reliable results across different operators, instruments, and sample types. Reproducibility tests have shown that the enzyme performs consistently over multiple runs, ensuring reliability in diagnostic testing. For more details, please see **Section 11.**
- **Stability and Robustness:** This enzyme exhibits exceptional stability, maintaining its performance characteristics under a variety of storage conditions and usage cycles. This stability supports extended use in laboratory settings without compromising performance. Moreover, its robust formulation is capable of handling complex and multiple amplifications, effectively detecting a broad range of genetic targets even in challenging sample matrices. This makes it particularly effective for applications that require the amplification of multiple targets from semi-pure genetic material.

The enzyme's high concentration format and compatibility with lyophilization processes further enhance its utility in creating robust, sensitive, and specific diagnostic assays. This flexibility, when combined with the ability to customize assays with additional separate components, establishes Polaris® Taq Polymerase 50 U/µL, IVD, as the go-to enzyme for diagnostic laboratories, PCR kit manufacturers, and molecular testing facilities focused on crafting and deploying superior molecular diagnostics. The extensive validation and versatility of this enzyme underscores Polaris® Taq Polymerase 50 U/µL, IVD, as a robust class A diagnostic reagent, capable of accommodating varied diagnostic demands and capable of delivering reliable results across different biological matrices and testing conditions.

3. Intended Use

Polaris® Taq Polymerase 50 U/µL, IVD, is a validated Class A IVD reagent, intended for professional use in the real-time PCR detection of viral, bacterial and fungal nucleic acids in swab samples collected from patients. A positive result indicates the presence of pathogen nucleic acids but a clinical correlation with patient history and other diagnostic information is necessary to determine the patient's infection status. Negative results do not preclude microbial infection and should not be used as the sole basis for patient management decisions. Thus, the results obtained with this enzyme should be interpreted in conjunction with other clinical and laboratory findings. This IVD reagent is intended for use by properly licensed laboratory professionals. The performance characteristics of this product were established based on the detection of the respiratory viruses SARS-CoV-2, Influenza types A and B, and RSV, and other two highly relevant human pathogens, MRSA and HPV. Laboratories should validate performance for other targets according to local regulations and guidelines.

4. Principles of the Assay

The Polaris® Taq Polymerase 50 U/µL, IVD, leverages the established principles of polymerase chain reaction (PCR) combined with the specificity of the TaqMan probe-based detection system. The test follows a specific workflow:

1. **Amplification/Extension:** The Polaris® Taq Polymerase 50 U/µL, IVD, plays a pivotal role in extending primers to synthesize a new DNA strand complementary to the target cDNA (or DNA, in qPCR applications).
2. **TaqMan Probe Cleavage:** The enzyme has been developed to enable the degradation of TaqMan probes. TaqMan probes are oligonucleotide sequences labeled with a fluorescent reporter dye at one end and a quencher dye on the opposite end, designed to hybridize specifically to the target sequence between the forward and reverse PCR primers. During the PCR extension phase, Polaris® Taq Polymerase 50 U/µL, IVD, 5’ to 3’ exonuclease activity cleaves the probe, separating the reporter and quencher dyes. This separation results in the generation of a fluorescent signal by the reporter dye, which is proportional to the amount of target nucleic acid present in the sample.
3. **Real-Time Detection:** The assay should be conducted in real-time PCR machines capable of detecting and measuring the intensity of fluorescence emitted as the probes are degraded at each cycle. This fluorescence directly correlates with the amount of target nucleic acid amplified.

The integration of Polaris® Taq Polymerase 50 U/µL, IVD, into qPCR and RT-qPCR workflows offers a robust, efficient solution for the detection of nucleic acids across a broad spectrum of diagnostic applications. Its formulation, optimized for high performance and compatibility with the TaqMan probe-based system, simplifies assay setup, minimizes contamination risk, and guarantees consistent results. When used in conjunction with other essential reagents (refer to Section 7 for details on required materials and instrument specifications), Polaris® Taq Polymerase 50 U/µL, IVD, provides great flexibility and reliability for molecular diagnostics, making it an essential tool for detecting various pathogens and genetic markers in clinical samples.

5. Reagent Composition

The table below delineates the key components provided with Polaris® Taq Polymerase 50 U/µL, IVD, across different presentations:

COMPONENT		PRESENTATION	VOLUME	NUMBER OF TUBES/BOTTLES	NUMBER OF 20 µL REACTIONS
Polaris® Taq Polymerase 50 U/µL	**Taq 50 U/µL**	**MD06691**	0.35 mL	1	5 000
		MD06692	3.5 mL	1	50 000

Although Polaris® Taq Polymerase 50 U/µL, IVD, plays a pivotal role in the diagnostic test, a comprehensive pathogen detection assay involves several other components that are vital for optimized target nucleic acid sequence detection. It is imperative to note that certain necessary components for a complete reaction are not supplied alongside Polaris® Taq Polymerase 50 U/µL, IVD. Detailed information about these additional components is provided in **Section 7**.

6. Storage, Stability and Handling Conditions

The Polaris® Taq Polymerase 50 U/µL, IVD, is dispatched under conditions that include room temperature, blue ice, or dry ice to ensure its quality is maintained throughout transit. Upon receipt, it is imperative to adhere to the following storage and handling conditions to maintain the integrity and performance of the reagent:

- Immediate Storage: Upon arrival, promptly transfer the reagent to a storage environment between -85 °C and -15 °C. This temperature range is crucial for preserving the reagent's activity and stability.
- Minimizing Degradation: To minimize degradation, ensure the reagent is returned to the specified storage conditions immediately after use. Limiting exposure to room temperature is essential for maintaining reagent stability.
- Freeze-Thaw Cycles: The product is designed to withstand a minimum of 10 freeze-thaw cycles without significantly losing performance.
- Package Integrity: Inspect the package upon receipt. If the package is found to be damaged, contact NZYtech immediately for assistance. Prompt reporting of any issues is essential for ensuring you receive a product in optimal condition.
- Expiry Date: Adherence to the expiry date indicated on the product's packaging is crucial. Using the product beyond this date is not recommended, as it may affect the reliability of your test results. Ensure that disposal of the product post-expiry date complies with the procedures outlined in **Section 9.2**.

Following these guidelines will help ensure that the Polaris® Taq Polymerase 50 U/µL, IVD, remains effective for its intended use, providing reliable and accurate results in your diagnostic applications.

7. Materials and Equipment Required but Not Provided

To effectively use Polaris® Taq Polymerase 50 U/µL, IVD, users must obtain certain essential materials and reagents not included with the product. These additional components are necessary to ensure optimal performance and reliability of diagnostic assays. The following unprovided reagents are required to implement a diagnostic test with Polaris® Taq Polymerase 50 U/µL, IVD:

TEST COMPONENT/REAGENT REQUIRED BUT NOT PROVIDED	SKU
Polaris® Glycerol-free Taq Antibody 10 mg/mL, IVD	MD0673
Polaris® qPCR Buffer 2.5x, IVD	MD0686
Polaris® RT-qPCR Buffer 2.5x, IVD	MD0687
Polaris® dNTP mix 25 mM, IVD	MD0690
DEPC-treated water	-
Polaris® Reverse Transcriptase 285 U/µL, IVD	MD0777
Polaris® RNase Inhibitor 80 U/µL, IVD	MD0778
Primers and Probes from NZYtech Real-Time PCR CE-IVD kits*	**

** Visit the NZYTech portfolio at www.nzytech.com. Check the kit references in **Section 18**.*

The Polaris® Taq Polymerase 50 U/µL, IVD, has been validated for use with the primers and probes included in the following NZYtech IVD kits: High-Risk HPV Multiplex Real-time PCR Kit, IVD (NZYtech catalogue number: MD0492), MRSA Multiplex Real-time PCR Kit, IVD (NZYtech catalogue number: MD0493), COVID-19, Flu A/B, RSV Multiplex One-Step RT-qPCR, IVD (NZYtech catalogue number: MD0490) and SARS-CoV-2 One-Step RT-qPCR Kit III, 5 Targets, IVD (NZYtech catalogue number: MD0491). Users seeking to employ different primers and probes in conjunction with Polaris® Taq Polymerase 50 U/µL, IVD, must undertake a rigorous validation process to ensure compatibility and efficacy. The use of alternative primers and probes without proper validation may affect the assay's performance and reliability.

Other essential materials and equipment required but not provided are:

- Real-time PCR Instrument: Ensure the instrument can detect FAM™, HEX™/VIC™/JOE™, Texas Red®/JUN™ and Cy5™ fluorescent dyes (at emission wavelengths of 520, 556, 603 and 670 nm, respectively). Please refer to **Section 10** for a list of validated instrument models.
- DNA & RNA Isolation Equipment: Apparatus and consumables necessary for extracting nucleic acids from different specimens.
- RNase & DNase-free PCR Plasticware: Including PCR tubes, strips, caps, 96-well plates and adhesive films.
- Pipettors and Filter Tips: Ensure they are RNase & DNase-free.
- Disposable Gloves: To prevent contamination and ensure sample integrity.
- Vortex and Centrifuge: Essential for mixing and sample preparation.

By adhering to these recommendations and ensuring the use of validated materials and reagents, users can maximize the diagnostic potential of the Polaris® Taq Polymerase 50 U/µL, IVD. Compliance with IVD requirements is only ensured when this reagent is used following the rules and guidelines set out in this IFU.

8. Sample Collection and Preparation

Optimal results in PCR-based diagnostic tests hinge on strict adherence to best practices in sample collection, transport, storage, and processing from biological specimens. The handling of the samples from collection to analysis directly influences the integrity of the testing process and the accuracy of the results. Follow these guidelines to ensure the highest quality of samples for PCR testing:

- Prompt Testing: It is essential to process and test samples as soon as possible after collection. Delays can lead to RNA degradation, significantly affecting the accuracy of the test results.
- Transport and Storage: Samples must be transported and stored at low temperatures to preserve the integrity of the nucleic acids. Adherence to local biosafety and transportation regulations is mandatory to ensure the safe handling of potentially infectious materials.
- DNA, RNA or Total Nucleic Acid Extraction: The extraction of DNA, RNA or total nucleic acid must be conducted using a CE-IVD marked device/kit. This ensures that the extraction process meets the stringent quality and performance standards necessary for accurate diagnostic testing.
- Assessing Sample Quality: Before proceeding with qPCR, verify the suitability of the nucleic acid samples. Key factors to assess include:
  - Purity: Evaluate the purity of the RNA/DNA by measuring the absorbance ratio at 260/280 nm. Ratios between 1.8 and 2.1 generally indicate good purity.
  - Concentration: Use spectrophotometric analysis or an equivalent method to determine the nucleic acids concentration.
  - Integrity: Assess the integrity of the nucleic acids. Degraded samples may yield unreliable results. Agarose gel electrophoresis or equivalent methods can help determine the integrity of nucleic acids.

By meticulously following these sample collection and preparation guidelines, you can significantly enhance the reliability and accuracy of your PCR diagnostic tests. Proper handling from collection through to analysis is critical for achieving the best possible outcomes in molecular diagnostics.

9. Precautions and Warnings

Adhering to good laboratory practices and closely following the provided procedures and guidelines is imperative for the accurate performance of the test. Any deviation from the procedures may lead to assay failure or erroneous results.

9.1. Safety Information

Please consult the Safety Data Sheet (SDS) available on the NZYtech website (www.nzytech.com) before using this reagent. Only personnel trained in relevant technical and safety procedures should perform molecular diagnostic testing through PCR, and it must be conducted in suitably equipped laboratories. Follow all applicable international and national laboratory biosafety guidelines.

9.2. Handling and Procedural Requirements

- Professional Use: This reagent is intended for use by professional *in vitro* diagnostic use.
- Expiration date: Do not use this reagent post-expiry data and ensure the product seal is intact before use.
- DNase & RNase-free Requirements: Employ DNase & RNase-free disposable plasticware and pipettes, utilizing filter tips to prevent contamination.
- Working Areas: Maintain separate working areas for sample preparation, reaction set-up, and amplification to prevent contamination.
- Post-Testing Clean-up: Post-amplification, clean surfaces and equipment using a DNA/RNA remover (for example DNA & RNA Cleaner, NZYtech, catalogue number: MB462), and handle plates carefully, disposing of them into biohazard containers immediately after use.
- Biological Sample Handling: Treat all biological samples as potentially infectious and follow biosafety precautions.
- Waste Management: Comply with national and regional regulations for disposal of chemical residues and preparations, generally considered as hazardous waste.
- Result Interpretation: A healthcare professional should interpret all results considering the patient's medical history and clinical symptoms.
- Laboratory Practices: Ensure adherence to good laboratory practices. Always wear appropriate protective clothing, disposable powder-free gloves, goggles and a mask. Do not eat, drink or smoke in the working area.

10. Testing Procedure

To ensure the successful execution of a PCR assay using Polaris® Taq Polymerase 50 U/µL, IVD, adhere closely to the qPCR (refer to Section 10.1) or the RT-qPCR (refer to Section 10.2) protocols outlined below. Familiarize yourself with the necessary additional reagents as listed in **Section 7**. Given the complexity of the PCR process, strict compliance with the protocol and precise handling of all components are essential to maintain reagent integrity and avoid PCR artifacts that may affect detection sensitivity.

- Before starting the assay, gently vortex the tubes to ensure uniformity of the solution. Keep the tubes on ice throughout the preparation phase to preserve reagent stability.
- Assemble all reaction components on benchtop coolers or ice to minimize temperature fluctuations that could impact reagent efficacy.
- To prevent PCR artifacts and ensure the highest sensitivity, promptly proceed to the PCR step following plate preparation. Delays or exposure of the reaction mixture to room temperature could lead to the formation of artifacts.
- Employ rigorous pipetting techniques to prevent cross-contamination. It is particularly important to add the template nucleic acid/Positive Control last when setting up your plate to avoid contamination of other samples.

10.1. qPCR Reaction set-up

1. Prepare the qPCR mix by combining Polaris® Taq Polymerase 50 U/µL, IVD, with all necessary reagents for effective diagnostics. Calculate the volume of the enzyme mix required for your diagnostic tests. Use the following guide, where *n* denotes the total number of reactions:

COMPONENT	**1 TEST VOLUME (µL)**	***n* TESTS* VOLUME (µL)**
Polaris® Taq Polymerase 50 U/µL, IVD	0.07	*n* x 0.07
Polaris® Glycerol-free Taq Antibody 10 mg/mL, IVD	0.1	*n* x 0.1
Polaris® qPCR Buffer 2.5x, IVD	8	*n* x 8
Polaris® dNTP mix 25 mM, IVD	0.24	*n* x 0.24
DEPC-treated water	1.59	*n* x 1,59
Primer & Probe Mix 10x from a NZYtech CE-IVD kit**	2	*n* x 2
FINAL VOLUME	**12**	***n* x 12**

** Ensure to include two extra reactions for the No-Template Control and Positive Control in your total reaction count. To account for pipetting variations, we advise including an extra 5% in your volume calculations.*
*** Use primers and probes from kits specified in **Section 7**.*

2. Mix well and spin down.

3. Pipette 12 µL of the qPCR mix prepared above into individual wells, according to your real-time PCR experimental plate configuration.

4. For the No-Template Control, add 8 µL of NTC, instead of DNA template, into the no-template control well. The final volume should be 20 µL.

5. For the Clinical Samples, add 8 µL of each respective DNA sample into the sample wells, according to your experimental plate configuration. The final volume in each well should be 20 µL.

6. For the Positive Control, add 8 µL of the POS Control from one of the NZYtech's qPCR CE-IVD kits referred to in **Section 7**, instead of the DNA template, into the positive control wells. The final volume should be 20 µL.

7. Securely seal the plate with appropriate caps or optical adhesive film to prevent evaporation and contamination.

8. Place the sealed reaction plate in the real-time PCR instrument and proceed with the qPCR run according to the following specific instructions:

CYCLES	**TEMPERATURE**	**TIME**	**STEP**
1	95 °C	3 min	Polymerase activation
40	95 °C	5 s	Denaturation
	60 °C	30 s	Annealing/Extension*

** Fluorogenic data should be collected during this step through the channels described in the selected Real-Time PCR CE-IVD kit.*

This protocol was used to validate diagnostic assays on several Real-time PCR Systems, including Applied Biosystems® 7500 FAST, Applied Biosystems® StepOnePlus, Roche Life Science LightCycler® 480 II, Applied Biosystems® QuantStudio 6 Pro, Qiagen Rotor-Gene Q and Bio-Rad® CFX96™. However, these conditions may be adapted and validated to suit different machine-specific protocols.

10.2. RT-qPCR Reaction set-up

1. Prepare the RT-qPCR mix by combining Polaris® Taq Polymerase 50 U/µL, IVD, with all necessary reagents for effective diagnostics. Calculate the volume of the enzyme mix required for your diagnostic tests. Use the following guide, where *n* denotes the total number of reactions:

COMPONENT	1 TEST VOLUME (µL)	*n* TESTS* VOLUME (µL)
Polaris® Taq Polymerase 50 U/µL, IVD	0.07	*n* x 0.07
Polaris® Glycerol-free Taq Antibody 10 mg/mL, IVD	0.1	*n* x 0.1
Polaris® RT-qPCR Buffer 2.5x, IVD	8	*n* x 8
Polaris® dNTP mix 25 mM, IVD	0.24	*n* x 0.24
Polaris® Reverse Transcriptase 285 U/µL, IVD	0.22	*n* x 0.22
Polaris® RNase Inhibitor 80 U/µL, IVD	0.50	*n* x 0.50
DEPC-treated water	0.87	*n* x 0.87
Primer & Probe Mix 10x from a NZYtech One-Step CE-IVD kit**	2	*n* x 2
FINAL VOLUME	**12**	***n* x 12**

** Ensure to include two extra reactions for the No-Template Control and Positive Control in your total reaction count. To account for pipetting variations, we advise including an extra 5% in your volume calculations.*
*** Use primers and probes from kits specified in **Section 7**.*

2. Mix well and spin down.

3. Pipette 12 µL of the RT-qPCR mix prepared above into individual wells, according to your real-time PCR experimental plate configuration.

4. For the No-Template Control, add 8 µL of NTC, instead of RNA template, into the no-template control well. The final volume should be 20 µL.

5. For the Clinical Samples, add 8 µL of each respective RNA sample into the sample wells, according to your experimental plate configuration. The final volume in each well should be 20 µL.

6. For the Positive Control, add 8 µL of the POS Control from one of the NZYtech's qPCR CE-IVD kits referred to in **Section 7**, instead of the RNA template, into the positive control wells. The final volume should be 20 µL.

7. Securely seal the plate with appropriate caps or optical adhesive film to prevent evaporation and contamination.

8. Place the sealed reaction plate in the real-time PCR instrument and proceed with the qPCR run according to the following specific instructions:

CYCLES	TEMPERATURE	TIME	STEP
1	50 °C	10 min	Reverse Transcription
1	95 °C	2 min	Polymerase activation
40	95 °C	5 s	Denaturation
	60 °C	30 s	Annealing/Extension*

**Fluorogenic data should be collected during this step through the channels described in the selected Real-Time PCR CE-IVD kit.*

This protocol was used to validate diagnostic assays on several Real-time PCR Systems, including Applied Biosystems® 7500 FAST, Applied Biosystems® StepOnePlus, Roche Life Science LightCycler® 480 II, Applied Biosystems® QuantStudio 6 Pro, Qiagen Rotor-Gene Q and Bio-Rad® CFX96™. However, these conditions may be adapted and validated to suit different machine-specific protocols.

11. Performance Evaluation

The Polaris® Taq Polymerase 50 U/µL, IVD, has undergone a rigorous validation process following IVD regulatory standards to establish its performance as a Class A IVD product. This section details the experiments conducted and the data obtained during the performance evaluation, underscoring the product's efficacy, reliability, and compliance. This evaluation utilized the primer-probe mix along with both positive and negative controls from the following four CE-IVD kits: High-Risk HPV Multiplex Real-time PCR Kit, IVD (MD0492), MRSA Multiplex Real-time PCR Kit, IVD (MD0493), COVID-19, Flu A/B, RSV Multiplex One-Step RT-qPCR Kit, IVD (MD0490) and SARS-CoV-2 One-Step RT-qPCR Kit III, 5 Targets, IVD (MD0491).

11.1. Expected Results

The Polaris® Taq Polymerase 50 U/µL, IVD, was used to test clinical samples using the COVID-19, Flu A/B, RSV Multiplex One-Step RT-qPCR, IVD, Kit (MD0490). Figure 1 presents typical amplification plots for various clinical samples tested with Polaris® Taq Polymerase 50 U/µL, IVD: clinical negative samples (Figure 1A), samples from patients infected with SARS-CoV-2 (Figure 1B), samples from patients co-infected with SARS-CoV-2 and Influenza A/B (Figure 1C), and samples from patients co-infected with SARS-CoV-2, Influenza A/B, and RSV (Figure 1D).

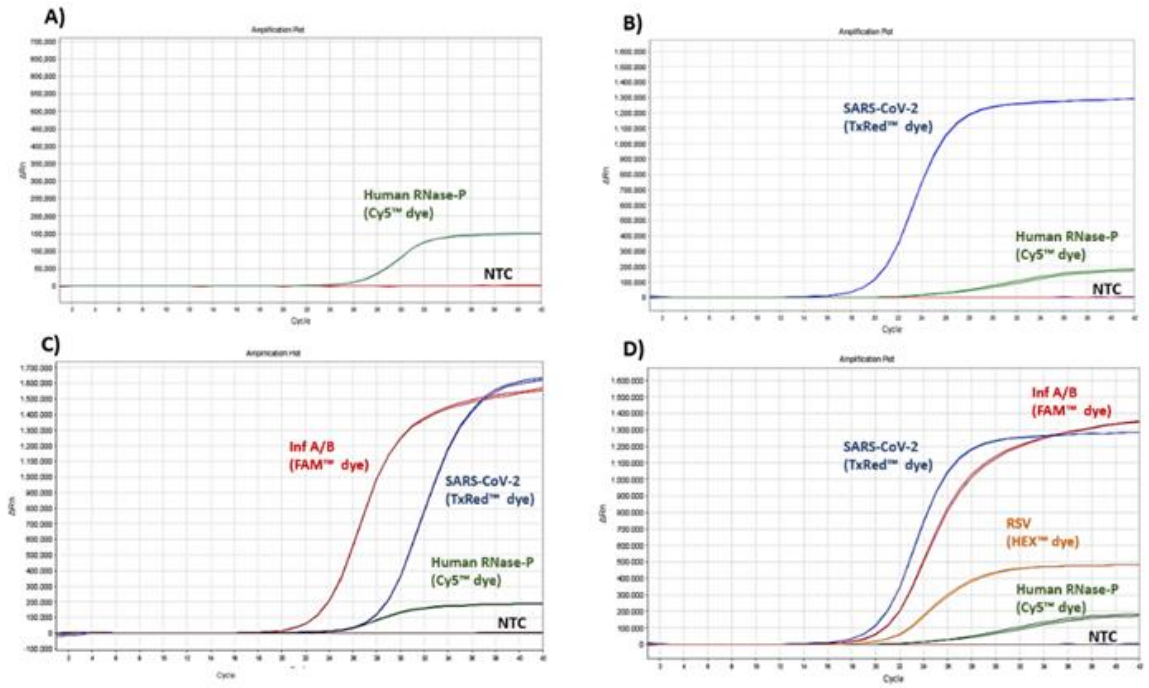

Figure 1. Using Polaris® Taq Polymerase 50 U/µL, IVD, to detect SARS-CoV-2, Influenza A/B, RSV and Human RNase-P targets from negative clinical samples (A), clinical samples infected with SARS-CoV-2 (B), co-infection with SARS-CoV-2 and Influenza A/B (C) and co-infection with SARS-CoV-2, Influenza A/B, and RSV (D). Blue curve, detection of the SARS-CoV-2 viral RNA targets through the Texas Red/JUN channel. Red curve, detection of Influenza A and/or Influenza B targets through the FAM channel. Orange curve, detection of the RSV L gene through the VIC/HEX channel. Green curve, detection of the human RNase P gene through the Cy5 channel.

11.2. Purity

The Polaris® Taq Polymerase 50 U/µL, IVD, is subjected to stringent purity assessments to ensure its reliable performance in advanced molecular diagnostic applications. To uphold the highest standards of quality and performance, key contaminants are rigorously tested across all production batches. The following table presents the purity assessment results, demonstrating the enzyme's compliance with rigorous purity criteria:

PURITY TEST	RESULT
SDS-PAGE (Protein purity/integrity)	>95% Pure
DNases	None detected
RNAses	None detected
E. coli DNA	≤1 Genome Copy/Reaction
16S Bacterial DNA	≤1 Genome Copy/Reaction
18S Human DNA	≤0.1 Genome Copy/Reaction

These purity assessments validate the Polaris® Taq Polymerase 50 U/µL, IVD, suitability for critical diagnostic applications, ensuring that it meets the exacting requirements essential for accurate and efficient molecular diagnostics. Rigorous testing for contaminants such as DNases, RNases, and unwanted genomic DNA ensures consistent high performance and specificity in nucleic acid synthesis and amplification.

11.3. Limit of Detection (LoD) - Analytical Sensitivity

The analytical sensitivity, or the lowest analyte concentration detectable with 95% confidence by the Polaris® Taq Polymerase 50 U/µL, IVD, was assessed across various conditions. Establishing the limits of detection (LoDs) involved detailed analysis, including spiking negative clinical matrices with known quantities of microbial nucleic acids. These LoDs were further refined through multiple testing rounds, ensuring both accuracy and repeatability. Initial experiments conducted over a minimum of three days using at least two different enzyme batches under typical testing conditions led to the provisional determination of LoDs. These preliminary findings, based on data obtained from samples containing varying genome copies for each microorganism, laid the groundwork for further verification. Confirmatory testing, conducted by two independent operators using at least three batches of the enzyme across 48 replicates, reinforced the preliminary LoDs. The overall LoDs for different testing scenarios were established as follows:

- High-Risk HPV Detection: Using the NZYtech High-Risk HPV Multiplex Real-time PCR Kit, IVD (MD04921), the enzyme proved its capability to detect HPV types across a range from 375 copies/mL (HPV39) to 2500 copies/mL (HPV33) among 14 different high-risk HPV genotypes.
- MRSA Detection: In concert with the NZYtech MRSA Multiplex Real-time PCR Kit, IVD (MD04931), the enzyme displayed the capability to detect 150 copies/mL of MRSA targets.

- Respiratory Virus Detection: Collaborative validation using the NZYtech COVID-19, Flu A/B, RSV Multiplex One-Step RT-qPCR Kit, IVD (MD04901), affirmed the enzyme's ability to detect SARS-CoV-2, RSV, and Influenza A/B at LoDs ranging from 250 to 375 copies/mL.
- Comprehensive SARS-CoV-2 Detection: With the NZYtech SARS-CoV-2 One-Step RT-qPCR Kit III, 5 Targets, IVD (MD04911), the enzyme was validated to specifically detect SARS-CoV-2 viral RNA at a LoD of 250 copies/mL.

Additionally, the analytical sensitivity in a co-infection scenario was tested by adding exactly 10^3 copies of each respiratory virus to the standard curves of the others, using mock co-infection specimens. This experiment was conducted with quadruplicate samples across three enzyme batches using the COVID-19, Flu A/B, RSV Multiplex One-Step RT-qPCR Kit, IVD (MD04901). The data revealed that while the LoD for SARS-CoV-2 remained stable, the LoD for RSV increased to 0.75 copies/µL (750 copies/mL), and for Influenza B to 1.25 copies/µL (1250 copies/mL). For Influenza A, the LoD rose to 2.5 copies/µL (2500 copies/mL) in co-infection conditions.

11.4. Interfering Substances

The impact of potentially interfering biological and chemical substances in the performance of Polaris® Taq Polymerase 50 U/µL, IVD, was assessed in tests consisting of negative specimens spiked with different pathogen specimens at ~3x LoD. These substances, which may be present in clinical specimens, include human whole blood, various antimicrobials, antifungal creams, washing products, and moisturizers, etc. 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 tests were performed at least in triplicate and results were compared to data obtained with a control test that contained no interferents. At the concentrations tested, the results revealed that none of the molecules under test affected the sensitivity of the detection. However, when tested for HPV, BETADINE® vaginal solution may cause interference at concentrations higher than 0.04% v/v. These data affirm the robustness of Polaris® Taq Polymerase 50 U/µL, IVD, when used in the context of different molecular testing scenarios, demonstrating resilience to a wide array of potential interferents. This ensures the reliability and accuracy of diagnostic results even in the presence of substances that might be encountered in clinical specimens. All experiments were run on the Applied Biosystems™ 7500 FAST Real-time PCR Instrument (used with 7500 software v2.3).

11.5. Precision

Precision studies for Polaris® Taq Polymerase 50 U/µL, IVD, were comprehensively conducted across various diagnostic assays to evaluate the enzyme's reliability and reproducibility in detecting different pathogens. These studies, pivotal for ensuring the enzyme's consistent performance, were performed employing the following NZYtech's kits: High-Risk HPV Multiplex Real-time PCR Kit, IVD; MRSA Multiplex Real-time PCR Kit, IVD; COVID-19, Flu A/B, RSV Multiplex One-Step RT-qPCR Kit, IVD; and SARS-CoV-2 One-Step RT-qPCR Kit III, 5 Targets, Kit, IVD. The assays targeted different pathogens at microbial load levels ranging from 3x to 30x the limit of detection (LoD), using DNA or RNA extracted from negative clinical specimens and spiked with known quantities of the target pathogen. Across all assays, precision was assessed through various metrics, including repeatability (variation within the same run), daily reproducibility (variation across different days), lot-to-lot reproducibility (variation between different kit batches), operator reproducibility (variation between different operators), and inter-instrument reproducibility (variation between different qPCR instruments). Positive samples were included in the studies and tested under standard reaction conditions, with the detection of each pathogen analyzed individually.

The findings consistently demonstrated 100% replicate detection across nearly all pathogens and testing conditions, indicating no significant variation that would impact the diagnostic outcome. The coefficient of variation (Cq) across different metrics remained low (<5%), underscoring the enzyme's reliable performance in the context of the different tests. Notably, even at varied microbial load levels, the enzyme's ability to accurately detect pathogens was not compromised, with Cq averages remaining within expected ranges. However, minor variations were observed in some metrics, such as the coefficient of variation in inter-instrument reproducibility, which varied slightly among different qPCR platforms. Despite these variations, the overall detection capability of Polaris® Taq Polymerase 50 U/µL, IVD, remained unaffected, with a consistent 100% detection rate across most conditions tested. Overall, these precision studies highlight the exceptional reliability of Polaris® Taq Polymerase 50 U/µL, IVD, in a range of diagnostic contexts, affirming its suitability for high-stakes molecular diagnostics. By maintaining high standards of repeatability and reproducibility, Polaris® Taq Polymerase 50 U/µL, IVD, ensures accurate pathogen detection, essential for effective disease management and control.

12. Clinical evaluation

The clinical performance of tests incorporating Polaris® Taq Polymerase 50 U/µL, IVD, was thoroughly assessed through validation studies across four diagnostic assays, incorporating a diverse array of clinical samples. These studies aimed to validate the enzyme's efficacy in the detection of various pathogens. Overall, the data can be summarized as:

- High-Risk HPV Detection: The evaluation involved 359 cervical swab samples analyzed using both isothermal PCR and real-time qPCR techniques. The study achieved 100% clinical sensitivity for HPV16 and HPV18, with specificity rates of 95% and 99% respectively, and 94% sensitivity alongside 100% specificity for other HR HPV types. Further comparison with commercial real-time PCR kits on 220 samples confirmed these high sensitivity and specificity levels, underscoring the enzyme's reliability in HPV detection.
- MRSA Detection: The validation analyzed 157 nasal swab samples characterized by routine microbiological culture. The MRSA Multiplex Real-time PCR Kit, IVD, displayed a clinical sensitivity of 98.28% and a specificity of 74.75%. Additional testing with a real-time PCR comparator and alternative nucleic acid extraction methods affirmed the enzyme's high sensitivity and specificity, with results indicating a minimal impact from differing sample processing techniques.
- Respiratory Virus Detection: A comprehensive study involving 1516 oro-nasopharyngeal swab samples tested for SARS-CoV-2, Influenza A/B, and RSV yielded a 99% agreement across all tested samples. Comparative analysis further validated the enzyme's high clinical sensitivity and specificity, achieving 100% in both metrics.
- SARS-CoV-2 Detection: The SARS-CoV-2 One-Step RT-qPCR Kit III, 5 Targets, IVD, underwent evaluation with 651 nasopharyngeal swab samples by an external laboratory, showcasing a clinical sensitivity of 97.4% and a specificity of 100%, confirming the enzyme's precision in detecting SARS-CoV-2.

These evaluations collectively demonstrate Polaris® Taq Polymerase 50 U/µL, IVD, exceptional performance across a range of clinical diagnostics, confirming its suitability for detecting significant pathogens with high accuracy. The validation studies highlight the enzyme's robust clinical utility, offering reliable results across different biological matrices and testing conditions. This unified clinical evaluation underscores the enzyme's integral role in molecular diagnostics, providing a solid foundation for its widespread application in clinical settings.

13. Quality Control and Product Stability

Polaris® Taq Polymerase 50 U/µL, IVD, is meticulously produced through rigorous quality control (QC) processes, all conducted under the stringent guidelines of ISO 13485:2016, to safeguard its consistent performance and reliability, and specifically according to the stipulations inherent to Class A IVD reagents. The final product is subjected to a cascade of validations to certify its superior performance, addressing aspects such as purity, functional performance, and the absence of pivotal contaminants. The stability of Polaris® Taq Polymerase 50 U/µL, IVD, was verified under a spectrum of storage conditions, ensuring that the enzyme retains its efficacy within the declared shelf-life. This assurance extends to both optimal and stress storage conditions, including product in-usage and shipping scenarios. Functional testing, underpinning the verification of product performance, is executed through a standardized testing protocol. This ensures that the enzyme operates optimally under practical application conditions and consistently yields reproducible results across different batches. Batch-to-batch consistency is meticulously quantified to guarantee minimal variability between diverse production batches. Furthermore, periodic checks and user feedback mechanisms are institutionalized to monitor the performance of Polaris® Taq Polymerase 50 U/µL, IVD, in real-world applications, thereby facilitating a continual and dynamic quality assurance process. As a testament to NZYtech's commitment to quality and accountability, Polaris® Taq Polymerase 50 U/µL, IVD, maintains complete traceability from production to storage and shipment. This traceability ensures accordance with the highest standards of quality throughout the entire lifecycle of the product.

14. 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.

15. Trademarks and Disclaimers

Polaris® is a registered trademark of NZYtech, Lda. All other trademarks, service marks, and logos appearing in this manual are the property of their respective owners.

16. 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

17. Conformity Declaration

Product name: Polaris® Taq Polymerase 50 U/µL, IVD

Catalogue number(s): MD06691 and MD06692

Intended use: intended for professional use in the real-time PCR detection of viral, bacterial and fungal nucleic acids in swab samples collected from patients

Classification: Class A

Manufacturer: NZYtech, Lda.

Estrada do Paço do Lumiar, Campus do Lumiar

Edifício E, R/C,

1649-038 Lisboa

Portugal

We, NZYtech, Lda, hereby declare that this product, to which this declaration of conformity relates, complies with the following standards and other normative documents ISO 9001:2015 and ISO 13485:2016, following the provisions of the Regulation (EU) 2017/746 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.

Joana Brás, PhD

Person responsible for regulatory compliance

18. References

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