Polaris® qPCR Pack

Catalogue number	Presentation
MD07821	200 rxns of 20 µL

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.

Features

- Includes HS Taq Polymerase (20 U/µL), qPCR Buffer (2.5x), dNTPs mix (25 mM), and DEPC-treated Water, comparable to Polaris® IVD class A reagents.
- Provides an assortment of qPCR reagents, similar to our class A IVD reagents, to facilitate effective optimization of novel qPCR applications.
- NZYtech class A IVD reagents (https://www.nzytech.com/en/molecular-diagnostics/mdx-reagents/polarisr-rt-qpcr-enzymes-additives/), similar to those supplied in this pack, have been extensively validated to detect various pathogens across multiple sample matrices.
- Supports large-scale production and validation, ensuring scalability and versatility for molecular biology laboratories

Description

The Polaris® qPCR Pack offers a comprehensive collection of reagents, meticulously designed to meet the complex needs of modern molecular diagnostics. This versatile toolkit is ideal for laboratories seeking to enhance assay performance across diverse testing applications. The pack includes essential reagents such as HS Taq Polymerase (20 U/µL), qPCR Buffer (2.5x), dNTPs mix (25 mM), and DEPC-treated Water, comparable to Polaris® IVD class A reagents. At the heart of this pack is the Polaris® HS Taq Polymerase (research version), known for its high activity unit concentration and designed to deliver the sensitivity and specificity required in complex molecular diagnostic testing. The enzyme, formulated with a 20 U/µL concentration and activity blocked by the highly efficient anti-Taq Polaris Taq Antibody, is ideal for the bulk production of PCR master mixes. The inclusion of 20% glycerol bolsters stability and freeze protection, yet its concentrated nature guarantees minimal glycerol integration, preserving the enzyme's suitability for lyophilization. NZYtech provides similar reagents in substantial quantities (https://www.nzytech.com/en/molecular-diagnostics/mdx-reagents/polarisr-rt-qpcr-enzymes-additives/) to support the scalability and versatility needed by molecular biology laboratories and PCR manufacturers, facilitating thorough validation and customization for specific applications. This package not only enables rigorous evaluation of the Polaris® HS Taq Polymerase and the other included reagents but also promotes the exploration of synergistic effects that can arise from combining compatible reagents, thereby improving the performance of molecular diagnostic assays. Suitable for detecting a variety of infectious diseases and genetic anomalies, the Polaris® qPCR Pack equips users with essential resources for innovating and refining qPCR tests, advancing research, and optimizing molecular diagnostics assay performance.

Shipping & Storage

This product is shipped from dry ice to blue ice. Upon arrival, promptly store all components at -85 °C to -15 °C in a constant temperature freezer. Avoid direct sunlight exposure. Immediately after use, return the components to a temperature between -85 °C and -15 °C to minimize exposure to room temperature. This product is stable through a minimum of 10 freeze-thaw cycles. Adhering to these meticulous storage procedures ensures that the Polaris® qPCR Pack will remain stable until the expiry date and deliver reliable and consistent performance in all applications.

Components

The Polaris® qPCR Pack provides all the essential reagents for performing 200 qPCR reactions, each with a final volume of 20 µL, as outlined in the table below. If individual reagents are needed, you can find their IVD versions on the NZYtech website (https://www.nzytech.com/en/molecular-diagnostics/mdx-reagents/polarisr-rt-qpcr-enzymes-additives/).

COMPONENT		TUBE/VIAL	VOLUME
Polaris® HS Taq Polymerase 20 U/µL	HS Taq 20 U/µL	1	35 µL
Polaris® dNTPs mix 25 mM	dNTP mix 25 mM	1	48 µL
Polaris® qPCR Buffer 2.5x	qPCR Buffer 2.5x	1	1.6 mL
DEPC-treated Water	DEPC-treated Water	1	1 mL

Reagents, Materials and Equipment Required but Not Provided

To successfully perform qPCR reactions using the Polaris® qPCR Pack, users will need to procure additional reagents, materials, and equipment that are not included in the pack. The list below outlines essential items required for qPCR assay setup, execution, and analysis but are not provided within the Polaris® qPCR Pack:

- **qPCR Primers and Probes:** Specific to the target of interest.
- **Positive Control Template:** A known quantity of target nucleic acid or a synthetic template ensures the qPCR reaction functions correctly.
- **RNase & DNase-free PCR Plasticware:** Including PCR tubes, strips, caps, 96-well plates and adhesive films.
- **Pipettors and Filter Tips:** Ensure that they are RNase & DNase-free.
- **qPCR Thermal Cycler:** Equipped with fluorescence detection capabilities for real-time data acquisition and analysis.
- **Vortex and Centrifuge:** Essential for mixing and reaction preparation.
- **Data Analysis Software:** Compatible with your qPCR thermal cycler for interpreting qPCR data.
- **Personal Protective Equipment (PPE):** Including gloves, lab coats, and eye protection to maintain safety and contamination control. Real-time PCR Instrument.

Ensure that all reagents and equipment used comply with the appropriate standards for molecular diagnostic use. Follow all relevant guidelines and manufacturer recommendations for handling and use.

Standard Protocol

Recommendations before starting.

- Handling instructions:
  - To help prevent any carry-over DNA contamination, you should assign independent areas for reaction set-up and qPCR amplification. Any tubes containing the amplified product must not be opened in the qPCR set-up area. Use sterile filtered tips.
  - All pipetting actions and experimental plate preparations must be diligently performed on benchtop coolers or ice to safeguard the integrity of the reagents and to mitigate the risk of generating qPCR artifacts, which could compromise the sensitivity and/or specificity of detection.
  - Upon plate preparation, swiftly progress to initiating the qPCR protocol.
- Reagent usage:
  - It is strongly recommended to thoroughly review the usage instructions of all involved reagents before assay execution.
  - Ensure homogeneity of the reagents before use. To achieve this, gently flick the tubes provided to homogenize the contents, then centrifuge for a few seconds to collect the volume at the bottom of the tube. Maintain tubes on ice.
  - Employ rigorous pipetting techniques to prevent cross-contamination. It is particularly important to add the Positive Control last when setting up your plate to avoid contamination of other samples.
  - Use the DEPC-treated water provided.
- Controls: To verify the absence of contamination, prepare a negative control reaction without a template (No-Template Control, NTC, or negative control). Additionally, include a Positive Control to serve as a reference for ensuring the correct functioning of the qPCR reaction and detection system. The positive control should exhibit the expected amplification and/or fluorescence signal, confirming the assay's ability to accurately detect the target sequence.

Procedure for qPCR testing

This standard protocol provides a foundational guideline for conducting qPCR reactions. While it serves as a reliable starting point, some parameters may require adjustments based on specific needs, such as reaction temperature or the initial quantity of template and, as such, this protocol can be adapted accordingly. The effectiveness of this suggested protocol is contingent on the proper storage and condition of all supplied components.

1. On ice, in a sterile nuclease-free microcentrifuge tube, prepare a reaction mixture, combining the following components (the volumes listed below are to prepare a single reaction mixture of 20 µL):
   Notes:
   - *Add water first and the remaining components in the order specified in the table below. The template should be the last component added, preferably in a separate work area.*
   - *To calculate the total number of reactions required per assay, consider the total number of samples to test and include two additional reactions to accommodate the No-Template and Positive Controls.*
   - *If setting up more than one reaction, prepare a reaction mixture volume 5% greater than the total required for the number of reactions to be performed.*

COMPONENT	1 TEST VOLUME (µL)	*n* TESTS VOLUME + 5% (µL)
DEPC-treated water	1.59	*n* x 1.669
Polaris® qPCR Buffer 2.5x*	8	*n* x 8.4
Polaris® dNTP mix 25 mM	0.24	*n* x 0.252
Polaris® HS Taq 20 U/µL	0.17	*n* x 0.1785
Primer & Probe Mix 10x	2	*n* x 2.1
FINAL VOLUME	**12**	***n* x 12.6**

** Polaris® Buffers at its 1x concentration, contain 2.5 mM $MgCl_2$.*

2. Mix and quickly pulse the reaction mixture.
3. For the No-Template Control (NTC), add 8 µL of NTC, instead of DNA template, into the no-template control well. The final volume should be 20 µL.
4. 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.
5. For the Positive Control, add 8 µL of the POS Control specific to the qPCR experiment, instead of the DNA template, into the positive control wells. The final volume should be 20 µL.
6. Securely seal the plate with appropriate caps or optical adhesive film to prevent evaporation and contamination.
7. Place the sealed reaction plate in the real-time PCR instrument and proceed with the qPCR run according to the specific instructions provided in the subsequent section.
8. Run the general qPCR protocol defined below in a real-time instrument for fluorescence-based real-time qPCR:

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

9. Store qPCR products at - 85 °C to -15 °C or directly proceed to downstream applications.

Technical Notes

Sample material

The accuracy and reliability of PCR-based tests, including those utilizing Polaris® HS Taq Polymerase 20 U/µL, heavily depend on the quality of the sample collection and preparation processes. Adhering to optimal procedures ensures the preservation of nucleic acids, maintaining their integrity for effective amplification and detection. Below are guidelines for collecting and preparing samples for analysis:

Sample Collection:

- **Type of Samples:** Collect appropriate clinical samples based on the suspected condition or target. Common types include oro-nasopharyngeal swabs for respiratory viruses, blood for systemic infections, and tissue.
- **Collection Devices:** Use sterile, nucleic acid-free collection devices to avoid contamination. Ensure that swabs, tubes, and other collection tools are compatible with downstream processing steps.

- **Handling and Transport:** Immediately after collection, place samples in a suitable transport medium if applicable (e.g., viral transport medium for swabs). Keep samples at 2-8 °C for short-term storage or transport and at -85 °C to -65 °C or lower for long-term storage, following guidelines specific to the sample type and anticipated delay before analysis.

Sample Preparation:

- **Nucleic Acid Extraction:** Extract nucleic acids using a method appropriate for the sample type and compatible with downstream PCR applications. Utilize extraction kits that are optimized for high yield and purity.
- **Quantification and Quality Assessment:** Quantify extracted nucleic acids using spectrophotometry or fluorometry to ensure adequate concentration for PCR. Assess the purity by evaluating the A260/A280 ratio, aiming for values between 1.8 and 2.0. Verify the integrity of DNA and RNA samples by gel electrophoresis or other suitable methods, especially for RT-qPCR applications (RNA).
- **Preparation for PCR:** Adjust the concentration of nucleic acids to meet the requirements of the PCR assay. Prepare aliquots to minimize freeze-thaw cycles and potential degradation if not used immediately.
- **Prevention of Contamination:** Employ best practices to prevent cross-contamination during sample handling and preparation. Use separate areas and sets of equipment for sample collection, nucleic acid extraction, and PCR setup. Implement the use of barrier (filtered) tips and wear appropriate protective gear.

Following these guidelines for sample collection and preparation is critical for achieving reliable and meaningful results with Polaris® HS Taq Polymerase 20 U/µL, in PCR-based diagnostics. Proper handling from the point of collection to the PCR setup ensures the integrity and usability of the samples, paving the way for accurate nucleic acid amplification and detection.

Primers and probe

These guidelines refer to the design and set-up of dual-labelled probes. Please refer to the relevant literature when using other probe types. The specific amplification, yield and overall efficiency of any real-time qPCR can be critically affected by the sequence and concentration of the probes and primers, as well as by the amplicon length. We strongly recommend considering the following points when designing and running your real-time qPCR experiment:

- **Primer and Probe Temperatures:** Aim for a primer melting temperature (Tm) of 58-62 °C, with the probe Tm approximately 10 °C higher than the primer's Tm.
- **Amplicon Length:** To ensure efficient amplification, the target amplicon should be 80-200 bp, not exceeding 300 bp.
- **Primer Concentration:** A 400 nM final concentration is generally effective for most probe-based assays. For optimal results, titrate between 150-600 nM, maintaining equimolar concentrations of forward and reverse primers.
- **Probe Concentration:** A final concentration of 200 nM is recommended for most cases. The probe concentration should be at least half that of the primers. Titrate between 50-300 nM to find the optimal concentration.
- **Multiplexing Considerations:** In multiplex qPCR, be mindful that high probe concentrations may lead to fluorescence bleed-over between channels. In addition, check all primers and probes involved in the multiplex test for cross-primer dimer formation.

Testing multiple primer-probe sets, provides a systematic approach to selecting the best primer-probe set, ultimately ensuring the success of molecular diagnostics applications.

Negative Control

Incorporating a negative control in your real-time qPCR tests is an essential practice for ensuring the accuracy and reliability of your results. A negative control, typically composed of all reaction components except the template DNA or RNA, helps to identify potential contamination or non-specific amplification. It serves as a critical baseline to distinguish true target detection from background noise. By validating the absence of unintended amplification, the negative control contributes significantly to the overall integrity of the experimental setup. Always include a negative control in each run to maintain stringent quality control and to affirm the specificity of your diagnostic test.

Positive control

The use of positive controls in real-time qPCR assays is crucial for verifying the assay's functionality and the accuracy of results. A positive control, which contains a known quantity of target nucleic acid or a synthetic analogue, confirms the efficacy of the amplification process and the detection system. It provides a benchmark for expected amplification efficiency and specificity, ensuring the reaction components are working as intended. Including a positive control in each experimental run is vital for assessing the assay's performance and for troubleshooting any unexpected outcomes.

Quality control assays

Purity

The enzyme included in the Polaris® qPCR Pack is >98% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Genomic DNA contamination

Polaris® qPCR Pack components are tested to verify that they contain ≤1 bacterial genome copy per reaction and ≤0.1 human genome copy per reaction. This is evaluated through qPCR detection.

Nucleases assay

To test for DNase contamination, 0.2-0.3 µg of pNZY28 DNA are incubated with each of the Polaris® qPCR Pack components for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with these components for 1 h at 37°C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

The components of Polaris® qPCR Pack are extensively tested for performance in qPCR experiments.

Troubleshooting

Troubleshooting qPCR assays requires a systematic approach, adjusting one variable at a time to identify and resolve any issues. The following recommendations are designed to address common challenges encountered during qPCR amplification with the Polaris® qPCR Pack. These tailored suggestions combine specific adjustments and broader strategies to improve the efficacy of your troubleshooting efforts. For additional technical support or questions, NZYtech is ready to assist.

NO AMPLIFICATION DETECTED
• **Inappropriate storage conditions.**
Store the Polaris® qPCR Pack in a dark place, preferably in its original packaging, at temperatures between -85 °C to -15 °C. Minimize exposure to ambient light and avoid direct sunlight.
• **Excessive amount of sample in the reaction**
Ideally, the sample/template should not exceed 50% of the total reaction volume in qPCR. If inhibition is suspected due to high sample concentration, reduce the template volume to 1-5 µL per 20 µL reaction.
• **Suboptimal qPCR conditions**
Consider adjusting the annealing temperature or extending the extension time in your qPCR protocol to improve amplification. Verify that modifications do not introduce non-specific amplification.
• **Contamination with DNases**
Ensure all equipment, including pipettes and tubes, is free from DNase/RNase. Utilize DNase/RNase-free water and sterile labware. Regularly changing gloves and properly storing extracted samples at -85°C to -65°C can also mitigate enzyme degradation.
LOW AMPLIFICATION YIELD
• **Presence of inhibitors**
While Polaris® HS Taq Polymerase 20 U/µL is designed to be robust against inhibitors, high concentrations of certain substances can still hinder reaction efficiency. Ensure samples are purified, avoiding excessive volumes of potentially crude extracts.
PRESENCE OF NON-SPECIFIC BANDS
• **Primer and/or Probe mis-design**
Verify the design of the primers-probe sets and validate their ability to selectively amplify the desired DNA fragment. Poor qPCR primer design is responsible for most non-specific amplifications.
• **Cross-contamination**
Create separate work areas for sample processing and qPCR setup. Use separate, dedicated pipettes, and disposable tips for each setup. Always follow good laboratory practices to avoid contamination. Include negative controls (no DNA template) in qPCR reactions to monitor for contamination in reagents or labware.
AMPLIFICATION IN NTCs
• **Carryover Contamination**
Implement a strict workflow where post-PCR areas are physically separated from pre-PCR areas.
• **Primer-Dimer Formation**
Design primers using software that minimizes the potential for primer-dimer formation. Optimize the annealing temperature to reduce non-specific binding.

By methodically addressing these common qPCR challenges, users can significantly improve the outcomes of assays performed with the Polaris® qPCR Pack. Troubleshooting is iterative; diligent adjustments and observations are key. For ongoing difficulties, NZYtech's technical support team is available to provide further guidance and assistance.

Similar IVD Reagents:

If you are looking for individual reagents, you can find class A IVD reagents on the NZYtech website (https://www.nzytech.com/en/molecular-diagnostics/ivd-reagents/).

COMPONENT		SKU
Polaris® HS Taq Polymerase 20 U/µL, IVD	HS Taq 20 U/µL	MD0670
Polaris® dNTPs mix 25 mM, IVD	dNTP mix 25 mM	MD0690
Polaris® qPCR Buffer 2.5x, IVD	qPCR Buffer 2.5x	MD0686

Suitable for veterinary, agriculture, water, and pharmaceutical testing procedures. Do not use in human diagnostic (IVD) procedures.

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