MBPCR281 Hi-PCR® hMPV Real Time Probe PCR Kit

Description

Human metapneumovirus causes upper and lower respiratory disease in young children, older adults and people with weakened immune system. Clinical symptoms of HMPV infection ranges from common cold, fever, nasal congestion to serious complications like bronchitis or pneumonia. The incubation period of the virus is between 3 and 6 days and the duration of illness can vary depending on the severity of infection. Transmission of hMPV infection occurs through infectious airborne droplets. Spread of Human metapneumovirus normally peaks during winter at the same time as other common respiratory viruses. However, it continues to spread all year round. Research shows that about 10% to 12% of respiratory illnesses in children are caused by HMPV. It is estimated that 5 to 16% of these children can develop severe lower respiratory infection such as pneumonia.

HMPV belongs to Pneumoviridae family, it is an enveloped virus with non-segmented RNA. HMPV has been classified into group A and group B, which is further subdivided into A1, A2, B1, B2, respectively. Due to its possible complication in young children and elderly adults with weakened immune system, accurate diagnosis of hMPV infection is essential. Real-time reverse-transcription polymerase chain reaction (RT-PCR) is the widely used method in diagnosis of hMPV owing to its high specificity and sensitivity. **Hi-PCR® hMPV Real Time Probe PCR Kit** is developed to aid in early diagnosis and surveillance to monitor for outbreak of human metapneumovirus from human clinical samples.

NOTE: Hi-PCR® hMPV Real Time Probe PCR Kit is for *in-vitro* use only.

Intended Use: Hi-PCR® hMPV Real Time Probe PCR Kit is designed for sensitive and specific detection of the human metapneumovirus in a one-step assay i.e. reverse transcription and amplification are performed in same tube. The kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR procedures.

Principle

Hi-PCR® hMPV Real Time Probe PCR Kit is based on the principle of real-time PCR. The technique is designed to amplify targeted nucleic acid sequences using hydrolysis probes that are short oligonucleotides with a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. Hi-PCR® hMPV Real Time Probe PCR Kit allows detection of HMPV in a one-tube assay format. The assay includes primers and probes specific for HMPV with probes labeled with ROX fluorophore. Additionally, the kit incorporates an endogenous internal control (IC) amplification system (probe labelled with fluorophore Cy5) to ensure efficient PCR amplification.

Controls

Positive Control

A Positive control (PC) is a control reaction which contain the target DNA sequence that the PCR is designed to amplify. It is usually used to ensure proper and intended functioning of all the PCR reagents and is recommended to be used in every run to assess optimal assay performance.

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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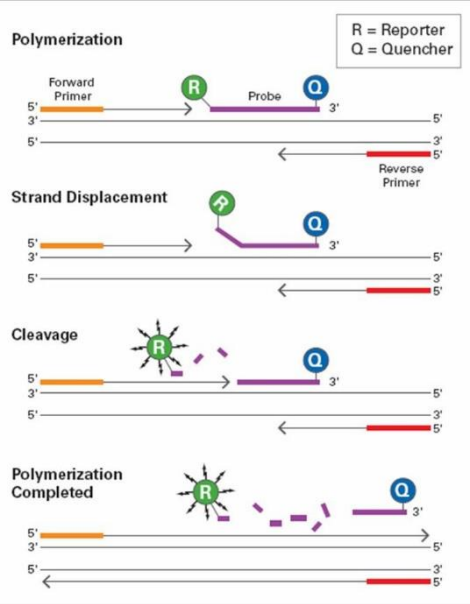
Negative Template Control

A Negative Template Control (NTC) is essential to verify that the reagents, equipment, and environment used in the assay are free from contamination with target nucleic acid. In this control reaction, nuclease-free water is used as the template. It is recommended to include at least one negative template control reaction per run to ensure the reliability of the results.

Internal Control

This is a control sequence which is amplified in the same reaction tube along with the target sequence but detected with a different primer-probe set. An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified, identify potential PCR inhibition, and ensuring the integrity of the reagents used in the assay.

Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

Strand displacement: When the probe is intact, the report dye emission is quenched.

Cleavage: During each extension cycle,the DNA polymerase cleaves the reporter dye from the probe

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'- nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Features

- Detection of HMPV in a single tube assay format.
- Fast and reliable results within 115 minutes.
- One-step assay i.e. reverse transcription and amplification are performed in same tube.
- Includes all necessary reagents and controls to ensure test validity
- High sensitivity and specificity for accurate detection.
- Compatible with any open 4-channel, 5-channel and 6-channel qPCR cyclers.
- Wet-lab assays validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® AG Real Time PCR Systems.

Sample Type: RNA extracted from **nasopharyngeal and oropharyngeal s**wabs

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -10°C and -20°C. Repeated thawing and freezing of PCR reagents should be avoided, not more than 5 freeze-thaw cycles, as this may reduce the sensitivity. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Exposure to light, heat or humidity may also affect the shelf life of certain kit components and should be avoided. Degradation of specimen/ extracted RNA can also hamper the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on pack.

Specimen Handling

When handling specimens for HMPV testing, it is essential to follow appropriate procedures to prevent contamination and ensure safe handling. After use, all contaminated materials must be sterilized by autoclaving before disposal. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with other body fluids. Safety guidelines may be referred in individual safety data sheets.

Kit Contents: The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)* (µL)		
		25R	50R	100R
hMPV Buffer Mix	DS2195	338	675	1325
hMPV Enzyme Mix	DS2196	27	54	106
hMPV Primer-Probe Mix	DS2197	14	27	53
hMPV Positive control	DS2204	59	119	238
Water	DS0440	59	119	238

***** For a 25 µL PCR reaction

Materials needed but not provided

All materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and equipment	
Insta Q96®AG Real time PCR System, 96 well block, 5 channels	MBLA027
Insta Q96®AG 6.0 Real time PCR System, 96 well block, 6 channels	MBLA028
Insta Q96® Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012
Insta Q48® M4 Real time PCR System, 96 well block, 4 channels	LA1023
TabSpin™ Microcentrifuge	LA1089/LA1090
Automated nucleic acid extraction system and materials	
Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Mag16, Insta NX® Mag16^{Plus}	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32^{Plus}	LA1096, MBLA019
Insta NX® Mag96	LA1097
Extraction Kits	
HiPurA® Pre-filled Plates for Viral Nucleic Acid Purification	MB582MPF16
HiPurA® Pre-filled Cartridges for Viral Nucleic Acid Purification	MB582PC16
HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Cartridges)	MB583PC16200
HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Plates)	MB583MPF16200

HiPurA® DNA/ RNA Purification Kit	MB583
Tubes, plates and other consumables	
Varivol II Micropipettes (Capacity: 0.5 to 10 µL/10 to 100 µL/200 to 1000 µL)	LA611/LA614/LA615
µPet Autoclavable Micropipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)	LA955/LA958/LA959/LA960
Q4Pet Autoclavable Micropipette (Capacity: 0.5 to 10 µL/10 to 100 µL/100 - 1000 µL)	MBLA009/MBLA011/MBLA008
Barrier Tips, Maximum capacity 10 µL	LA749A
Barrier Tips, Maximum capacity 200 µL	LA751A
Barrier Tips, Maximum capacity 1000 µL	LA859A
8-strip tubes & optically clear flat caps for PCR	PR17, PR22, PR23
PCR Tubes, 0.1mL, 0.2 mL; PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18

Kit compatibility with Real-Time PCR Systems

Hi-PCR® hMPV Real Time Probe PCR Kit contains fluorophores that are compatible to the following PCR systems:

Real-Time PCR system	Company	Dye 1	Dye 2
Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/ Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	ROX	Cy5
BioRad CFX Opus 96/CFX96 Touch/ CFX384 Touch	Bio-Rad Laboratories, Inc.	Texas Red/ROX	Cy5
QuantStudio™ 5 / Quant Studio™ 6 and 7 Flex Real-Time PCR Systems / QuantStudio™ Dx	Applied Biosystems	ROX	Cy5
ABI® Prism SDS 7500	Applied Biosystems	Texas Red/ROX	Cy5
QIAquant 96 & 384 5plex	QIAGEN	Texas Red/ROX	Cy5
Rotor-Gene®6000 & Q	QIAGEN	Orange	Red
LightCycler® 96	Roche	ROX/Texas Red	Cy5
LightCycler® 480	Roche	ROX/Texas Red	Cy5
qTOWER^3	Analytik Jena	ROX/Texas Red	Cy5

Note: Ensure that the Real-Time PCR system is calibrated for dyes mentioned above and maintained according to the manufacturer´s instructions and recommendations.

Warning and Precautions

Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

General Preparation Instructions

- Before use all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous nucleic acid templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

Protocol for PCR Reaction Mix Preparation

1. In the "Master mix Preparation" area, thaw all components from the kit on ice, mix by inverting the tubes and centrifuge the reagents for 5 seconds. Keep on ice for later use.
2. Based on the total number of specimens (including PC and NTC) to be tested (N), calculate the volume of the components to be added as **N X volume of "1R"**
3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the PCR reaction mix. Refer the following table. After all the reagents are added, mix them thoroughly and centrifuge for 5 seconds.

Components	**Product code**	**Volume for "1R" (one reaction)**
Preparation of PCR Reaction Mix		
hMPV Buffer Mix	DS2195	12.5 µL
hMPV Enzyme Mix	DS2196	1.0 µL
hMPV Primer-Probe Mix	DS2197	0.5 µL
Total PCR Reaction Mix	-	**14.0 µL**
Template addition		
Template (Extracted RNA)		11.0 µL
Total reaction volume	**-**	**25.0 µL**

4. Aliquot 14 µL of PCR reaction mix into 0.1/0.2mL PCR tube/plate/strips, compatible to the PCR instrument to be used.
5. In the "Nucleic acid handling" area, add 11 µL of extracted nucleic acid of test specimen into the plate/strip to respective wells.
6. For positive and negative template control, template nucleic acid is replaced by Positive control mix and nuclease free water respectively. Refer the following table.

Set up of **Positive controls (PC)** for the PCR run		
Components	**Product code**	**Volume for "1R" (one reaction)**
Total PCR Reaction Mix	-	14.0 µL
hMPV Positive control	DS2204	11.0 µL
Total reaction volume	**-**	**25.0 µL**

Set up of **Negative controls (NC)** for the PCR run		
Components	**Product code**	**Volume for "1R" (one reaction)**
Total PCR Reaction Mix	-	14.0 µL
Water	DS0440	11.0 µL
Total reaction volume	**-**	**25.0 µL**

7. Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film.
8. Centrifuge the tube briefly at 6000 rpm for about 10 seconds.
9. Place the tubes in Real-time PCR machine and set the recommended PCR program (mentioned below).

Interpret the data from the amplification plot (observe the Ct values).

Recommended PCR program:

Sr. No	Step	Temperature	Time	Sampling	No. of cycles
1.	Reverse Transcription	55°C	20 minutes	---	1
2.	Initial denaturation	95°C	2 minutes	---	1
3.	Denaturation	95°C	15 seconds	---	45
4.	Annealing	55°C	45 seconds	YES	
5	Extension	72°C	15 seconds	---	

Selection of channels:

Target	Channels	Quencher
hMPV	Texas Red/ROX	None
IC	Cy5	None

Please select 'Passive reference dye' as 'None' wherever applicable

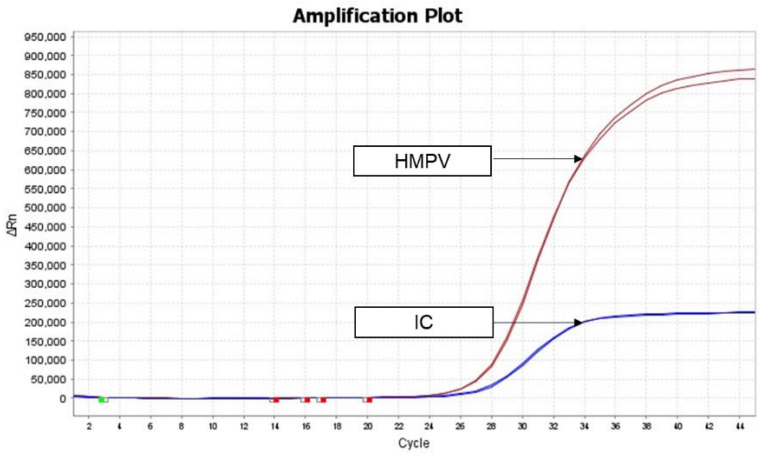
Data Analysis

The following conditions should be met for a valid diagnostic test:

Control	ROX (hMPV)	CY5 (IC)
Positive Control	+	+
Negative Template Control	-	-

Target	Ct value	Result/Interpretation
hMPV	≤ 37	Detected (+)

Amplification plot:



Sr. No	Targets	Ct value	
		PC	NTC
1	hMPV	26.64	--
2	IC	26.85	--

Note: Image representing probe based Real-Time amplification of hMPV and IC targets (Ct values provided in table are for representation) run on QuantStudio 5.

Data Interpretation:

ROX (hMPV)	CY5 (IC)	Result Interpretation
+	+/-	Positive for hMPV*
-	+	Negative for hMPV
-	-	Inconclusive test** Likely poor extraction or sample quality. PCR inhibition or reagent failure.

*The presence or absence of a signal in the ROX channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.
** When an inconclusive result is obtained, repeat PCR or re-test the extracted RNA or re-extract the specimen and test with PCR.

Note: All negative findings must be correlated with clinical observations. Negative results do not exclude infections because of other variants of these viruses.

Performance Evaluation

Analytical Sensitivity - Limit of Detection (LoD)
The Limit of Detection (LoD) is defined as the concentration (copies per µl of the eluate) of target molecule that can be detected at 95% or greater probability according to CLSI EP17-A2. The LoD assay of the Hi-PCR® hMPV Real Time Probe PCR Kit was performed using 20 replicates each on Biorad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96®AG Real time PCR System using Quantitative nucleic acids for human metapneumovirus (lineage B1) procured from Vircell. The detectable limit of Hi-PCR® hMPV Real Time Probe PCR Kit was determined to be 10 copies/µL for hMPV.

Analytical Specificity
Inclusivity
The ability of the Hi-PCR® hMPV Real Time Probe PCR Kit to detect a wide range of related target organisms has been assessed in the inclusivity parameter by two ways (i) *in silico* analysis of the oligonucleotides (primers and probes) and (ii) wet lab testing using nucleic acids of related target organisms. The oligonucleotide sequences of all the targets were checked by sequence comparison against all the relevant sequences of hMPV available in the GenBank database.

Exclusivity / Cross-Reactivity Analysis
The ability of the Hi-PCR® hMPV Real Time Probe PCR Kit to distinguish the target organisms from similar but genetically distinct non-target organisms has been assessed by (i) *in silico* analysis of the oligonucleotides (primers and probes) and (ii) wet lab testing using nucleic acids of non-related target organisms.

Wet lab testing of the Hi-PCR® hMPV Real Time Probe PCR Kit for potential cross-reactivity was performed using DNA/RNA from various pathogens available in the laboratory, on Applied Biosystems QuantStudio 5. None of the pathogens listed in the table below exhibited any reactivity with the primers and probes of the Hi-PCR® hMPV Real Time Probe PCR Kit.

Heat-inactivated SARS-CoV-2 (ATCC: 1986HK)	*Quantitative Synthetic Human coronavirus HKU1 RNA (ATCC: 3262SD)*
Quantitative Genomic RNA from Human rhinovirus 16 strain 11757 (ATCC: 283DQ)	Quantitative Genomic DNA from Human adenovirus 1 strain Adenoid 71 (ATCC: 1DQ)
Quantitative Genomic DNA from Human herpesvirus 5 (HHV-5) strain AD-169 (Cytomegalo virus) (ATCC: VR-538DQ)	Betacoronavirus 1 strain OC43 (ATCC: 1558DQ)
Human coronavirus 229E (ATCC: VR-740DQ)	Quantitative Genomic RNA from Enterovirus 68 strain Fermon (ATCC: 1826DQ)
Quantitative Genomic RNA from Human parainfluenza virus 1 strain C35 (ATCC: VR-94DQ)	Quantitative Genomic RNA from Human parainfluenza virus 2 strain Greer (ATCC: VR-92DQ)
Quantitative Genomic RNA from Human parainfluenzavirus 3 strain C 243 (ATCC: VR-93DQ)	Quantitative Genomic DNA from *Bordetella pertussis* (ATCC: 9797DQ)
Quantitative Genomic DNA from *Streptococcus pyogenes* strain Bruno (ATCC: 19615DQ)	Quantitative Genomic DNA from *Haemophilus influenzae* (ATCC: 51907DQ)
Quantitative Genomic DNA from *Pseudomonas aeruginosa* strain PAO1-LAC (ATCC: 47085DQ)	Quantitative Genomic DNA from *Staphylococcus aureus* subsp. Aureus (ATCC: 43300DQ)

Quantitative Genomic DNA from *Chlamydophila pneumoniae* strain CM-1 (ATCC: 1360DQ)	Quantitative Genomic DNA from *Mycoplasma pneumoniae* strain M129-B7 (ATCC: 29342DQ)
Quantitative Genomic DNA from *Legionella pneumophila* subsp. Pneumophila (ATCC: 33152DQ)	Quantitative Genomic DNA from *Mycobacterium tuberculosis* strain H37Ra
Quantitative Genomic DNA from Measles virus strain Edmonston (ATCC: VR-24D)	Quantitative Genomic RNA from Influenza A virus (H1N1) strain A/Virginia/ATCC1/2009 (ATCC: 1726DQ)
Quantitative Genomic RNA from Influenza B virus (ATCC: VR-1804DQ)	Quantitative Genomic RNA from Human respiratory syncytial virus strain 18537 (ATCC: 1580DQ)
Influenza A virus (H3N2) strain A/ Wisconsin/15/2009 (ATCC: VR-1882DQ)	Genomic DNA from *Corynebacterium diphtheriae* strain NCTC 13129 (ATCC: 700971D-5)

Evaluation

Each lot of Hi-PCR® hMPV Real Time Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of Hi-PCR® hMPV Real Time Probe PCR Kit has been functionally tested in amplification assay.

General Precautions

Strict compliance with the Instructions for Use is required for optimal results and the use of the kit is limited to staff qualified clinical laboratory personnel trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

This assay must not be performed on the specimen directly. RNA extraction should be performed using appropriate nucleic acid extraction method.

Presence of PCR inhibitors and other interferences may lead to false negative or invalid results.

Limitations

Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in failure to detect the presence of pathogen.

As with any diagnostic test, results of the Hi-PCR® hMPV Real Time Probe PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	Check the integrity of nucleic acid using agarose gel electrophoresis.
			Use freshly prepared RNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Check whether all components are added in correct volume as per the manual.
		Inappropriate storage conditions	Store the reagents at recommended temperature for its optimal performance. Check expiry of the reagents and use new lot of reagents if necessary.
		Incorrect PCR programming	Ensure selection of appropriate fluorescence channel as detailed in the manual. Compare the PCR program to the manual.
2.	Variability between replicates	Error in reaction set-up	Prepare a large volume master mix, vortex thoroughly and aliquot into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a Real-Time PCR instrument.
		Pipetting error	C_t values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes. Use calibrated pipettes. Repeat the run.
3.	Amplification of pathogen targets in Negative template control	Cross contamination during handling	Replace all critical solutions. Repeat the analysis of all tests with fresh aliquots of critical reagents. Follow good laboratory practices to avoid contamination issues.

Safety Information

Hi-PCR® hMPV Real Time Probe PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

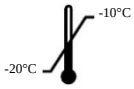
Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com

Symbols:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIMBPCR281
Rev.No.:00
Date of Issue: 2025-06