

## MBPCR206

## Hi-PCR<sup>®</sup> Astrovirus Probe PCR Kit

### Description

Astrovirus are non-enveloped, positive sense, single-stranded RNA viruses. It is the cause of 2 to 9% of all acute nonbacterial gastroenteritis viral diarrhea followed by nausea, vomiting, fever, malaise and abdominal pain in children worldwide. Moreover, Astrovirus may cause severe and sometimes fatal systemic and central nervous system (CNS) infections such as astrovirus-associated encephalitis and meningitis in immunocompromised individuals. It has also been shown to infect a wide range of hosts from birds to mammals. The infection is transmitted essentially through the fecal-oral route, contaminated food/water. The release of astroviruses into the environment is a concern due to the extreme stability of the virus (90 days in both marine and tap water). Nucleic acid amplification-based assays or Polymerase Chain Reaction (PCR) is a predominantly used method that allows for sensitive and specific detection of astrovirus RNA from clinical and environmental samples. Real-Time PCR technique is considerably simple and fast with respect to the standard PCR technique. This technique has been successfully used for the rapid detection and identification of a variety of infectious and non-infectious pathogens and genes.

**NOTE:** HiMedia's Hi-PCR<sup>®</sup> Astrovirus Probe PCR Kit is for *in-vitro* use only.

### Intended Use

Hi-PCR<sup>®</sup> Astrovirus Probe PCR Kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR and in vitro diagnostic procedures. The kit is recommended for sensitive and specific detection of Astrovirus RNA in water and clinical samples.

### Product Description:

Hi-PCR<sup>®</sup> Astrovirus Probe PCR Kit is based on real-time PCR technology for the detection of Astrovirus specific RNA. The kit contains primer-probe mixture specific for the detection of Astrovirus RNA. In addition, the kit contains a positive control and internal control for identification of possible PCR inhibition or reagent failure. The assay principle is based on the hydrolysis probe chemistry which confers higher specificity and sensitivity.

### Positive control

This is a control reaction using a known template (target pathogen). A positive control is usually used to ensure proper and intended functioning of all the PCR reagents and is recommended to be included in every run to assess optimal performance.

### Negative Control

A Negative control is needed to ensure that the reagents, equipment, and environment used in the assay is not contaminated. In this reaction, Nuclease free water is used as the template. It is recommended to have minimum one reaction of negative control per run.

### Principle

Real-time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. HiMedia's Hi-PCR<sup>®</sup> Astrovirus Probe PCR Kit is designed to detect the Astrovirus **RNA in FAM channel** with **Internal Control in JOE channel** in a single tube reaction. The kit allows sensitive and specific detection of Astrovirus in a single tube reaction.



#### Registered Office

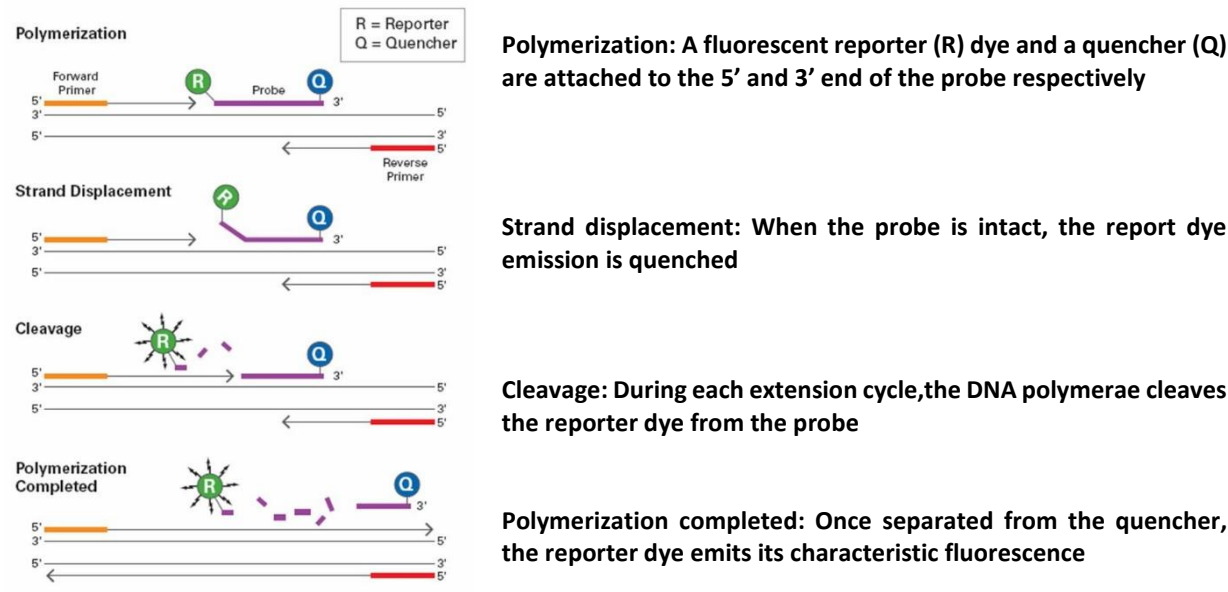
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## Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



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

- Fast and reliable results within 75 minutes.
- Includes all reagents and controls for the validity of the test.
- Open system – Compatible with any 3-channel, 4-channel and 5-channel qPCR cyclers.
- Good sensitivity and specificity.
- Wet-lab assays validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems.

**Sample Source:** Water and clinical samples

### Specimen Handling

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with blood. Safety guidelines may be referred in individual safety data sheets.

### Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -10°C to -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. Degradation of sample RNA specimens can also reduce the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on pack.

**Kit Contents:** The provided PCR kit contains:

Components	Product code	Reagents provided for* (µL)	
		25R	50R
Astrovirus Master Mix	DS2387	270	540
Enzyme Mix	DS2388	21.6	43.2
Astrovirus Primer-Probe Mix	DS2389	32.4	64.8
Molecular Biology Grade Water for PCR	ML065	43	86
Astrovirus Positive Control	DS2390	43	86

\* For a 20 µL PCR reaction

**Materials needed but not provided**

- Appropriate real-time PCR instrument.
- Appropriate nucleic acid extraction system or kit.
- Centrifuge with a rotor for 1.5ml - 2 ml reaction tubes.
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates.
- Vortex mixer.
- PCR tubes (0.1ml or 0.2ml) or 96 well reaction plates with corresponding (optical) closing material or lid.
- Pipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL).
- Pipette tips with filters (As per pipette capacity).
- Powder-free gloves (disposable).

**Materials needed but not provided**

All materials are available through [www.himedialabs.com](http://www.himedialabs.com)

Product name	Product Code
<b>Real-Time PCR Instruments and other equipment</b>	
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
Insta Q48® M2 Real time PCR System, 96 well block, 2 channels	LA1024
TabSpin™ Microcentrifuge	LA1089/LA1090
HiPer® Mini Plate Centrifuge	LA1099
<b>Automated nucleic acid extraction system and materials</b>	
Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Mag16, Insta NX® Mag16 <sup>Plus</sup>	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32 <sup>Plus</sup>	LA1096, MBLA019
<b>Extraction Kits</b>	
HiPurA® Water Nucleic Acid Purification Kit	MB622
HiPurA® Water Viral RNA Purification Kit (with Nitrocellulose Membrane Filter)	MB620A
HiPurA® Water Viral RNA Purification Kit	MB620
HiPurA® Pre-filled plates for Water Nucleic Acid Purification Kit	MB622MPF16
HiPurA® Pre-filled Cartridges for Water nucleic Acid Purification (Insta NX® Mag16)	MB622PC16

HiPurA® Pre-filled plates for Water Nucleic Acid Purification Kit (Insta NX® Mag32)	MB622MPF-32
HiPurA® Pre-filled plates for Waste Water Nucleic Acid Purification Kit	MB622MPF-32A
HiPurA® Viral DNA/RNA Purification Kit	MB582
<b>Tubes, plates, and other consumables</b>	
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, 100µl Max capacity 100 µL	LA1104A
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, 0.1 mL, 0.2 mL; PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18
1.5 ml nuclease free Micro centrifuge tubes	PW146

#### Kit compatibility with Real-Time PCR Systems

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

Real-Time PCR system	Company	Dye 1 (Astrovirus)	Dye 2 (IC)
Insta Q96® AG/ Insta Q96® AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	FAM	JOE
QuantStudio™ 3 and 5 / Quant Studio™ 6 and 7 Flex Real-Time PCR Systems / QuantStudio™ Dx	Applied Biosystems	FAM	JOE
Applied Biosystems 7500	Applied Biosystems	FAM	JOE
BioRad CFX Opus 96/CFX96	Bio-Rad Laboratories, Inc.	FAM	JOE
Rotor-Gene® Q/QIAquant	QIAGEN	Green	Yellow
Roche LightCycler® 96	Roche	FAM	JOE
AriaMx	Agilent	FAM	JOE
Alta RT-96/48	Athenese-Dx Private Limited	FAM	JOE
qTOWER <sup>3</sup> auto	Analytik Jena	FAM	JOE

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

#### 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 RNA 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 number of specimens to be tested (N), calculate the volume of the components to be added as N X (volume of "1X")
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 "1X" (One Reaction)
<b>Preparation of PCR Reaction Mix</b>		
Astrovirus Master Mix	DS2387	10.0 µL
Enzyme Mix	DS2388	0.8 µL
Astrovirus Primer-Probe Mix	DS2389	1.2 µL
<b>Total PCR Reaction Mix</b>		<b>12.0 µL</b>
<b>Template addition</b>		
Purified Viral RNA	-	8.0 µL
<b>Total reaction volume</b>	-	<b>20.0 µL</b>

4. Aliquot 12.0 µL of PCR reaction mix into respective labeled 0.1/0.2mL PCR tube/plate/strips, compatible to the PCR instrument to be used.
5. In the "Nucleic acid handling" area, add 8.0 µL of extracted RNA of test specimen into the plate/strip to respective wells.
6. For setting up positive and negative control reactions, Viral RNA (template) is replaced by Astrovirus Positive Control and nuclease free water respectively. Refer the following table.

<b>Set up of controls for the PCR run</b>			
Components	Product code	Volume for "1X" (One Reaction)	
		Positive Control	Negative Control
Total PCR Reaction Mix	-	12.0 µL	12.0 µL
Astrovirus Positive Control	DS2390	8.0 µL	-
Water	ML065	-	8.0 µL
<b>Total reaction volume</b>	-	<b>20.0 µL</b>	<b>20.0 µL</b>

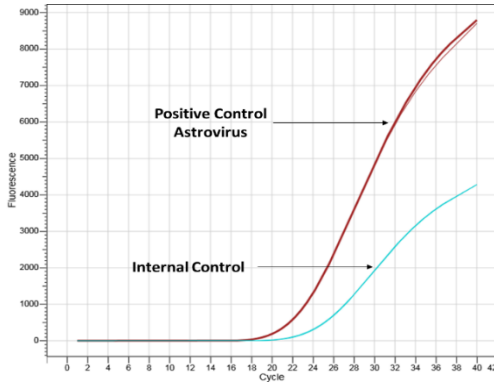
7. Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film.
8. Centrifuge the tube briefly at 6000 rpm for about 10 seconds.
9. Place the tubes in Real-time PCR machine and set the recommended PCR program (mentioned below). Interpret the data from the amplification plot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

**A. Recommended PCR program**

- |                          |   |                                |                     |
|--------------------------|---|--------------------------------|---------------------|
| 1. Reverse Transcription | : | 50°C for 15 minutes            |                     |
| 2. Initial denaturation  | : | 95°C for 2 minutes 30 seconds  |                     |
| 3. Denaturation          | : | 95°C for 15 seconds            |                     |
| 4. Annealing & Extension | : | 60°C for 30 seconds (Sampling) | } No. of cycles: 40 |
| Channels                 | : | FAM/JOE <sup>#</sup>           |                     |
| 5. Hold                  | : | 4°C for ∞                      |                     |

<sup>#</sup>For instruments not calibrated for JOE, HEX/VIC can be used.

**B. Amplification Data**



Sr No.	Sample	Ct Value
1.	Astrovirus Positive Control	22.61
2.	Internal Control	24.68

The image shows an amplification plot for the Astrovirus and internal control (IC) gene with associated Ct values generated by using Hi-PCR® Astrovirus Probe PCR Kit on InstaQ 96 series instrument (The Ct values provided in the table are for representation only).

**C. Data Analysis:**

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

Control	Detection channel	
	FAM (Astrovirus)	JOE (Internal Control)
Positive Control	+	+/-*
Negative Control	-	+

**D. Data Interpretation:**

Detection Channel		Result Interpretation
FAM (Astrovirus)	JOE (Internal Control)	
+	+/-*	Astrovirus Specific RNA detected
-	+	Astrovirus specific RNA is not detected. Sample does not contain detectable amounts of Astrovirus specific RNA.
-	-	PCR Inhibition or reagent failure. Retest the sample.

Ct value	Result
≤ 36	Detected (+)
> 36 or N/A	Not detected (-)

\*Detection of the internal control (IC) in the JOE channel is not necessary for confirming positive results in the FAM channel. Presence of high Astrovirus RNA load and/or PCR inhibitors in the original sample can result in a diminished or absent IC signal.

**Performance Evaluation**

**Limit of Detection (LoD) - Analytical Sensitivity**

The analytical sensitivity was defined as the lowest concentration of the target that could be reliably detected with 95% confidence. The analytical sensitivity for the Hi-PCR® Astrovirus Probe PCR Kit was conducted using synthetic DNA on InstaQ96® Real Time PCR system. The preliminary LoD of each target was determined by testing a 10-fold dilution series in triplicates per concentration, and then confirmed with 20 replicates of the concentration determined to be the detectable LoD. The data revealed that the Hi-PCR® Astrovirus Probe PCR Kit detects ≈ 3 copies/μL. Thus, the detectable Limit of Detection (LoD) was determined to be 3 copies/μL or

3000 copies/ml. The sensitivity analysis of the Hi-PCR® Astrovirus Probe PCR Kit was carried out on HiMedia's InstaQ 96 series, Biorad's CFX Series and Thermo Fisher's QuantStudio™ 5 Real-Time PCR System.

**Inclusivity - Analytical Sensitivity**

*In silico* analysis for the assessment of inclusivity for the Hi-PCR® Astrovirus Probe PCR Kit was conducted by mapping the primers and probes against all the available sequences in GenBank. The Hi-PCR® Astrovirus Probe PCR Kit targets 100% of the known Astrovirus strains.

**Analytical Reactivity**

The analytical reactivity of the Hi-PCR® Astrovirus Probe PCR Kit was verified through wet lab testing of the oligonucleotides (primers and probes) against commercially available Astrovirus control- ATCC Quantitative Synthetic RNA from Astrovirus (3238SD).

**Cross-reactivity - Analytical Specificity**

Wet testing analysis was performed against the pathogens available in the laboratory. In addition, *in silico* analysis was performed using NCBI nucleotide and Primer BLAST. The primers and probe for Astrovirus were analyzed against the viruses related to Astrovirus, organisms causing similar symptoms as an infection with Astrovirus and organisms with similar route of transmission. Below mentioned table represents the list of pathogens analyzed for analytical specificity. No cross-reactivity was observed with any strains mentioned below.

Campylobacter jejuni (ATCC BAA 1153)	Legionella pneumophila (33152DQ)	Hepatitis E Virus (VR-3258SD)
Chikungunya (VR-3246SD)	Giardia (30888D)	Enterovirus (VR-1826DQ)
Cryptosporidium parvum (PRA-67DQ)	Staphylococcus aureus (43300DQ)	Rotavirus (VR-2018DQ)
Escherichia coli (10798DQ)	Klebsiella pneumoniae (BAA ATCC 2146)	Adenovirus (VR-1DQ)
Streptococcus pneumoniae (ATCC 49619)	Pseudomonas aeruginosa (47085DQ)	Dengue Serotype 1 (VR-3228SD)
Enterococcus faecalis (ATCC 29212)	Staphylococcus epidermidis (12228DQ)	Dengue Serotype 2 (VR-3229SD)
Plasmodium falciparum (PRA-405)	ECO157 (NCTC 13846)	Dengue Serotype 3 (VR-3230SD)
Salmonella typhi (ATCC 14028)	JC Polyomavirus (VR-1583DQ)	Dengue Serotype 4 (VR-3231SD)
Shigella flexneri (ATCC 12022)	Rhinovirus (VR-283DQ)	Genomic DNA extracted from Vibrio cholerae
Leptospira (BAA-1198D-5)	Hepatitis A Virus (VR-3257SD)	

**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 samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product. 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. This assay must not be performed on the specimen directly. Viral RNA should be extracted from sample 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 under quantitation or failure to detect the presence of the target regions in these cases. Validity and performance of the assay design are revised at regular intervals.

### Performance and Evaluation

Each lot Hi-PCR® Astrovirus Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

### Quality Control

Each lot of HiMedia's Hi-PCR® Astrovirus Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. It has been functionally tested in DNA amplification assays.

### Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	Check the integrity of RNA using agarose gel electrophoresis. Use freshly prepared RNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions and storage conditions have been used.
		Error in reaction set-up	Prepare a large volume master mix, vortex thoroughly and aliquot into reaction tubes.
2.	Variability between replicates	Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a real-time PCR instrument.
		Pipetting error	C <sub>t</sub> values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes.
		Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.
3.	Amplification in negative control	Incorrect programming of the temperature profile of the thermocycler	Compare the temperature profile to the manual.

### Safety Information

HiMedia's Hi-PCR® Astrovirus 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 infectious sample must be decontaminated and disposed of in accordance with current laboratory techniques.

## 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](mailto: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.: PIMBPCR206

Rev.No.:00

Date of Issue: 2025-07

### Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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