

Instructions for Use

RealStar[®] hMPV RT-PCR Kit 2.0

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RealStar®

hMPV RT-PCR Kit 2.0

For use with

Mx 3005P™ QPCR System (Stratagene)
VERSANT® kPCR Molecular System AD (Siemens Healthcare)
ABI Prism® 7500 SDS (Applied Biosystems)
ABI Prism® 7500 Fast SDS (Applied Biosystems)
Rotor-Gene® 6000 (Corbett Research)
Rotor-Gene® Q5/6 plex Platform (QIAGEN)
CFX96™ Real-Time PCR Detection System (Bio-Rad)
CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
LightCycler® 480 Instrument II (Roche)



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Content

1. Intended Use	6
2. Kit Components	6
3. Storage	6
4. Material and Devices required but not provided	7
5. Background Information	8
6. Product Description	9
6.1 Real-Time PCR Instruments	10
7. Warnings and Precautions	11
8. Procedure	12
8.1 Sample Preparation	12
8.2 Master Mix Setup	13
8.3 Reaction Setup.....	15
9. Programming the Real-Time PCR Instrument	16
9.1 Settings	16
9.2 Fluorescence Detectors (Dyes).....	16
9.3 Temperature Profile and Dye Acquisition	17
10. Data Analysis	18
10.1 Validity of Diagnostic Test Runs	18
10.1.1 Valid Diagnostic Test Run.....	18
10.1.2 Invalid Diagnostic Test Run	18
10.2 Interpretation of Results	19
10.2.1 Qualitative Analysis	19

11. Performance Evaluation	20
11.1 Analytical Sensitivity.....	20
11.2 Analytical Specificity.....	22
11.3 Precision	23
12. Limitations	24
13. Quality Control	24
14. Technical Assistance	25
15. Literature	25
16. Trademarks and Disclaimers	25
17. Explanation of Symbols	26

1. Intended Use

The RealStar® hMPV RT-PCR Kit 2.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of human metapneumovirus (hMPV) specific RNA. Furthermore, the test allows the differentiation between hMPV subtype A (hMPV A) and hMPV subtype B (hMPV B) specific RNA.

2. Kit Components

Lid Color	Component	Number of Vials	Volume [μ l/Vial]
Blue	Master A	8	60
Purple	Master B	8	180
Green	Internal Control	1	1000
Red	Positive Control hMPV A	1	250
Orange	Positive Control hMPV B	1	250
White	Water (PCR grade)	1	500

3. Storage

- The RealStar® hMPV RT-PCR Kit 2.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact Altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between $+2^{\circ}\text{C}$ and $+8^{\circ}\text{C}$ should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE



Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer's instructions and recommendations.



It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).

5. Background Information

Human metapneumovirus (hMPV) are negative sense, single stranded RNA viruses of the family *Paramyxoviridae*. Like *human respiratory syncytial virus* (RSV), hMPV belong to the subfamily *Pneumovirinae*. Human metapneumoviruses are classified in the genus *Metapneumovirus* and subdivided into two major genetic lineages, hMPV A and hMPV B. Different genotypes of both subgroups can cocirculate within one epidemia. Human metapneumovirus is a common respiratory virus causing upper and lower respiratory tract infections with symptoms very similar to those caused by RSV.

Symptoms range from mild rhinorrhea to severe respiratory illness with bronchiolitis and pneumonia, the latter particularly in infants, young children, elderly, persons with cardiopulmonary diseases and immunocompromised individuals. The virus is spread by infectious droplets or by contact with nasal or oral secretions from infected people. Outbreaks of hMPV infections occur predominantly in winter and spring in temperate climates, often overlapping or following RSV outbreaks.

NOTE



Due to the relatively fast molecular evolution of RNA viruses, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results.

6. Product Description

The RealStar® hMPV RT-PCR Kit 2.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and differentiation of Human metapneumovirus A (hMPV A) and Human metapneumovirus B (hMPV B) specific RNA. The assay includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit.

Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

Probes specific for hMPV A RNA are labelled with a fluorophore showing similar characteristics to Cy[®]5 whereas the probes specific for hMPV B RNA are labelled with the fluorophore FAM[™]. The probe specific for Internal Control (IC) is labelled with the fluorophore JOE[™].

Using probes linked to distinguishable dyes enables the parallel detection of hMPV A and hMPV B specific RNA as well as the detection of the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of three processes in a single tube assay:

- Reverse transcription of target and Internal Control RNA to cDNA
- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® hMPV RT-PCR Kit 2.0 consists of:

- Two Master reagents (Master A and Master B)
- Internal Control (IC)
- Two Positive Controls:
 - Positive Control hMPV A
 - Positive Control hMPV B
- PCR grade water

Master A and Master B contain all components (PCR buffer, reverse transcriptase, DNA polymerase, magnesium salt, primers and probes) to allow reverse transcription, PCR mediated amplification and target detection of hMPV A specific RNA, hMPV B specific RNA and Internal Control in one reaction setup.

6.1 Real-Time PCR Instruments

The RealStar® hMPV RT-PCR Kit 2.0 was developed and validated to be used with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)

7. Warnings and Precautions

Read the Instructions for Use carefully before using the product.

- Before first use check the product and its components for:
 - Integrity
 - Completeness with respect to number, type and filling (see chapter 2. Kit Components)
 - Correct labelling
 - Frozenness upon arrival
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) sample preparation, (ii) reaction setup and (iii) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.

- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

8. Procedure

8.1 Sample Preparation

Extracted RNA is the starting material for the RealStar® hMPV RT-PCR Kit 2.0.

The quality of the extracted RNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are suitable for nucleic acid extraction:

- QIAamp® Viral RNA Mini Kit (QIAGEN)
- QIAasymphony® (QIAGEN)
- NucliSENS® easyMag® (bioMérieux)
- MagNA Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

Alternative nucleic acid extraction systems and kits might also be appropriate.

The suitability of the nucleic acid extraction procedure for use with RealStar® hMPV RT-PCR Kit 2.0 has to be validated by the user.

If using a spin column based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

CAUTION



If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.



The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

8.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® hMPV RT-PCR Kit 2.0 contains a heterologous Internal Control (IC), which can either be used as a RT-PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

- ▶ If the IC is used as a RT-PCR inhibition control, but not as a control for the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Internal Control	1 µl	12 µl
Volume Master Mix	21 µl	252 µl

- ▶ If the IC is used as a control for the sample preparation procedure and as a RT-PCR inhibition control, add the IC during the nucleic acid extraction procedure.
- ▶ No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the specimen/lysis buffer mixture.
- ▶ If the IC was added during the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	20 µl	240 µl

CAUTION

If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.



No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.

8.3 Reaction Setup

- ▶ Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ▶ Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the control (Positive or Negative Control).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	30 µl

- ▶ Make sure that each Positive Control and at least one Negative Control is used per run.
- ▶ Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.
- ▶ Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- ▶ Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

9. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument. For detailed programming instructions regarding the use of the RealStar® hMPV RT-PCR Kit 2.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

9.1 Settings

- Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX™

9.2 Fluorescence Detectors (Dyes)

- Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
hMPV A specific RNA	hMPV A	Cy [®] 5	(None)
hMPV B specific RNA	hMPV B	FAM™	(None)
Internal Control	IC	JOE™	(None)

9.3 Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Reverse Transcription	Hold	1	-	55	20:00
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	00:15
			yes	55	00:45
			-	72	00:15

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® hMPV RT-PCR Kit 2.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run

For a **valid** diagnostic test run, the following control conditions must be met:

Control ID	Detection Channel		
	Cy [®] 5	FAM [™]	JOE [™]
Positive Control hMPV A	+	-	+/-*
Positive Control hMPV B	-	+	+/-*
Negative Control	-	-	+

* The presence or absence of a signal in the JOE[™] channel is not relevant for the validity of the test run.

10.1.2 Invalid Diagnostic Test Run

A diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.2 Interpretation of Results

10.2.1 Qualitative Analysis

Detection Channel			Result Interpretation
Cy [®] 5	FAM [™]	JOE [™]	
+	-	+*	hMPV A specific RNA detected.
-	+	+*	hMPV B specific RNA detected.
-	-	+	Neither hMPV A nor hMPV B specific RNA detected. The sample does not contain detectable amounts of hMPV A or hMPV B specific RNA.
-	-	-	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE[™] detection channel is not required for positive results either in the Cy[®]5 detection channel or in the FAM[™] detection channel. High hMPV A and/or hMPV B RNA load/s in the sample can lead to reduced or absent Internal Control signals.

11. Performance Evaluation

Performance evaluation of the RealStar® hMPV RT-PCR Kit 2.0 was done using quantified hMPV A RNA (hMPV 27 Type A2, ZeptoMetrix) and hMPV B RNA (hMPV 3 Type B1, ZeptoMetrix).

11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® hMPV RT-PCR Kit 2.0 is defined as the concentration (copies per μl of the eluate) of hMPV A or hMPV B specific RNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of hMPV A and hMPV B specific RNA of known concentration.

Table 1: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of hMPV A specific RNA

Input Conc. [copies/ μl]	Number of Replicates	Number of Positives	Hit Rate [%]
50.000	24	24	100
31.622	24	24	100
10.000	24	24	100
3.162	24	24	100
1.000	24	22	92
0.316	24	22	92
0.100	24	10	42
0.032	24	5	21
0.010	24	0	0
0.003	24	0	0

Table 2: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of hMPV B specific RNA

Input Conc. [copies/ μl]	Number of Replicates	Number of Positives	Hit Rate [%]
50.000	24	24	100
31.622	24	24	100
10.000	24	24	100
3.162	24	24	100
1.000	24	22	92
0.316	24	10	42
0.100	24	6	25
0.032	24	1	4
0.010	24	0	0
0.003	24	0	0

The analytical sensitivity of the RealStar® hMPV RT-PCR Kit 2.0 was determined by Probit analysis:

- For the detection of hMPV A specific RNA, the analytical sensitivity is 0.68 copies/ μl [95% confidence interval (CI): 0.41 - 1.56 copies/ μl]
- For the detection of hMPV B specific RNA, the analytical sensitivity is 1.68 copies/ μl [95% confidence interval (CI): 1.02 - 3.73 copies/ μl]

11.2 Analytical Specificity

The analytical specificity of the RealStar® hMPV RT-PCR Kit 2.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant hMPV genotypes will be detected.

The analytical specificity of the RealStar® hMPV RT-PCR Kit 2.0 was evaluated by testing a panel of genomic RNA/DNA extracted from viruses related to hMPV and other pathogens causing similar symptoms as hMPV

The RealStar® hMPV RT-PCR Kit 2.0 did not cross-react with any of the following pathogens:

- Human adenovirus 1
- Human adenovirus 2
- Human adenovirus 3
- Human adenovirus 4
- Human parainfluenza virus 1
- Human parainfluenza virus 2
- Human parainfluenza virus 3
- Human parainfluenza virus 4a/b
- Human respiratory syncytial virus A
- Human respiratory syncytial virus B
- Influenza A virus H1N1nv
- Influenza A virus
- Influenza B virus
- Enterovirus, Coxsackie A3
- Rhinovirus
- Human coronavirus 229E
- *Bordetella pertussis*
- *Bordetella parapertussis*
- *Mycoplasma pneumoniae*
- *Haemophilus influenzae*
- *Legionella pneumophila*
- *Moraxella catarrhalis*
- *Streptococcus pneumoniae*

11.3 Precision

Precision of the RealStar® hMPV RT-PCR Kit 2.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the three analysis.

The variability data are expressed in terms of standard deviation and coefficient of variation based on threshold cycle (C_t) - values. At least six replicates per sample were analysed for intra-assay variability, inter-assay and inter-lot variability.

Table 3: Precision data for hMPV A and hMPV B specific RNA

hMPV A and hMPV B		Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	hMPV A	32.15	0.08	0.20
	hMPV B	32.11	0.03	0.10
Inter-Assay Variability	hMPV A	32.21	0.09	0.28
	hMPV B	32.17	0.07	0.22
Inter-Lot Variability	hMPV A	32.29	0.16	0.49
	hMPV B	32.33	0.13	0.40
Total Variability	hMPV A	32.31	0.19	0.59
	hMPV B	32.26	0.15	0.46

Table 4: Precision data for the detection of the Internal Control

Internal Control	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	29.52	0.13	0.43
Inter-Assay Variability	28.89	0.65	2.25
Inter-Lot Variability	28.63	0.39	1.36
Total Variability	28.92	0.54	1.85

12. Limitations

- Strict compliance with the instructions for use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of RT-PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- Potential mutations within the target regions of the hMPV A and hMPV B genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- Depending on the hMPV isolates and the real-time PCR instrument used for the analysis, slight cross reactivity might occur between the hMPV A and hMPV B system.
- As with any diagnostic test, results of the RealStar® hMPV RT-PCR Kit 2.0 need to be interpreted in consideration of all clinical and laboratory findings.

13. Quality Control

In accordance with the Altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of RealStar® hMPV RT-PCR Kit 2.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For technical advice, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

15. Literature

Versalovic, James, Carroll, Karen C., Funke, Guido, Jorgensen, James H., Landry, Marie Louise and David W. Warnock (ed). Manual of Clinical Microbiology. 10th Edition. ASM Press, 2011.

Cohen, Jonathan, Powderly, William G, and Steven M Opal. Infectious Diseases, Third Edition. Mosby, 2010.

16. Trademarks and Disclaimers

RealStar® (Altona Diagnostics); ABI Prism® (Applied Biosystems); ATCC® (American Type Culture Collection); CFX96™ (Bio-Rad); Cy® (GE Healthcare); FAM™, JOE™, ROX™ (Life Technologies); LightCycler® (Roche); Maxwell® (Promega); Mx 3005P™ (Stratagene); NucliSENS®, easyMag® (bioMérieux); Rotor-Gene®, QIAamp®, QIASymphony® (QIAGEN); VERSANT® (Siemens Healthcare).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

















The RealStar® hMPV RT-PCR Kit 2.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.

Not available in all countries.

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17. Explanation of Symbols

	<i>In vitro</i> diagnostic medical device
	Batch code
	Cap color
	Product number
	Content
	Number
	Component
	Global trade identification number
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Note
	Version

Notes:

always a drop ahead.

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