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Instructions for Use

RealStar®
EHEC PCR Kit 2.0

01/2017 EN

RealStar®

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EHEC PCR Kit 2.0

For research use only!

(RUO)

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1. Application

The RealStar® EHEC PCR Kit 2.0 is a qualitative reagent system, based on real-time PCR technology, for the detection and differentiation of DNA specific for Shiga toxin 1 (*stx1*) and Shiga toxin 2 (*stx2*) of *Escherichia coli* and the invasion plasmid antigen H (*ipaH*) of enteroinvasive *Escherichia coli*(EIEC) and *Shigella* spp..

For research use only (RUO)! Not for use in diagnostic procedures.

2. Kit Components

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

3. Storage

- The RealStar® EHEC 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°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Product Description

The RealStar® EHEC PCR Kit 2.0 is a qualitative reagent system, based on real-time PCR technology, for the detection and differentiation of DNA specific for Shiga toxin 1 (*stx1*) and Shiga toxin 2 (*stx2*) of *Escherichia coli* and the invasion plasmid antigen H (*ipaH*) of enteroinvasive *Escherichia coli* (EIEC) and *Shigella spp.*. The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit.
The Shiga toxin variants *stx1c*, *stx1d*, *stx2f* and *stx2ev*, which have not been shown to be associated with severe disease, cannot be detected with this test.

Real-time PCR technology utilizes 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 *stx1* DNA are labelled with a fluorophore showing similar characteristics to Cy®5, probes specific for *stx2* DNA are labelled with the fluorophore FAM™ and probes specific for *ipaH* DNA are labelled with the fluorophore ROX™. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of *stx1*, *stx2* and *ipaH* specific DNA, as well as the detection of the Internal Control in corresponding detector channels of the real-time PCR instrument.

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

- PCR amplification of target DNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® EHEC PCR Kit 2.0 consists of:

- Two Master reagents (Master A and Master B)
- Internal Control (IC)
- Positive Control [*stx1* + *stx2*+ *ipaH*]
- PCR grade water

Master A and Master B contain all components (PCR buffer, DNA polymerase, magnesium salt, primers and probes) to allow PCR mediated amplification and target detection of *stx1* specific DNA, *stx2* specific DNA, *ipaH* specific DNA and Internal Control in one reaction setup.

4.1 Real-Time PCR Instruments

The RealStar® EHEC PCR Kit 2.0 can 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)
- LightCycler® 480 Instrument II (Roche)

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

5. Procedure

5.1 Sample Preparation

Extracted DNA is the starting material for the RealStar® EHEC PCR Kit 2.0.

The quality of the extracted DNA 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® DNA Mini Kit (QIAGEN)
- QIAsymphony® (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.

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.***

CAUTION	
	***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 8. Technical Assistance).

5.2 Master Mix Setup

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

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

► If the IC is used as a 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 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 sample. The IC should always be added to the sample/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 sample/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 sample.***

5.3 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).

6. 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® EHEC PCR Kit 2.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 8. Technical Assistance).

6.1 Settings

► Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	None

6.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
stx1 specific DNA	*stx1*	Cy®5	(None)
stx2 specific DNA	*stx2*	FAM™	(None)
ipaH specific DNA	*ipaH*	ROX™	(None)
Internal Control (IC)	IC	JOE™	(None)

6.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	0:15
			yes	58	0:45

7. 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® EHEC PCR Kit 2.0 on different real-time PCR instruments please contact our Technical Support (see chapter 8. Technical Assistance).

7.1 Interpretation of Results

7.1.1 Qualitative Analysis

Detection Channel				Result Interpretation
Cy®5	FAM™	ROX™	JOE™	
+	-	-	+*	*stx1* specific DNA detected.
-	+	-	+*	*stx2* specific DNA detected.
+	+	-	+*	*stx1* and *stx2* specific DNA detected.
-	-	+	+*	*ipaH* specific DNA detected.
+	-	+	+*	*stx1* and *ipaH* specific DNA detected.
-	+	+	+*	*stx2* and *ipaH* specific DNA detected.
-	-	-	+	Neither *stx1*, nor *stx2* nor *ipaH* specific DNA detected. The sample does not contain detectable amounts of these specific DNAs.
-	-	-	-	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, FAM™ detection channel or in the ROX™ detection channel. A high target DNA load in the sample can lead to reduced or absent Internal Control signal.

8. Technical Assistance

For technical advice, please contact our Technical Support:

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

9. 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).

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For research use only! Not for use in diagnostic procedures.

10. Explanation of Symbols

RUO	For research use only
LOT	Batch code
CAP	Cap color
REF	Product number
CONT	Content
NUM	Number
COMP	Component
	Version
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Note

Notes:

always a drop ahead.

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