altona DIAGNOSTICS

Instructions for Use

RealStar®
EHEC PCR Kit 2.0

01/2017 EN

RealStar®

RealStar®

EHEC 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)

LightCycler® 480 Instrument II (Roche)

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Content

1. Intended Use

The RealStar® EHEC PCR Kit 2.0 is a qualitative *in vitro* diagnostic test, 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..

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

Enterohemorrhagic *Escherichia coli* (EHEC) are human pathogenic bacteria characterized by the ability to produce Shiga toxin 1 and/or Shiga toxin 2. Symptoms of an EHEC infection may include hemorrhagic colitis and the hemolytic uremic syndrome (HUS). EHEC strains may posess only one or both of these Shiga toxin coding genes (*stx1* and/or *stx2*). The gene coding for Shiga toxin 1 (*stx1*) is also present in *Shigella dysenteriae* type 1, which may cause the same symptoms as EHEC. Not all genetic variants of Shiga toxin 1 and Shiga toxin 2 are associated with severe disease, e.g. *stx1c*, *stx1d*, *stx2f* and *stx2ev* **[1, 2, 3, 4]**. By means of the marker "invasion plasmid antigen H" (*ipaH*) *Shigella* spp. can be distinguished from EHEC. *ipaH* is also present in enteroinvasive *Escherichia coli* (EIEC), that do not posess either of the Shiga toxin coding genes but can also cause bloody diarrhea.

Accurate diagnosis of EHEC infection is essential for patient management, infection control and epidemiology.

[1] http://www.bfr.bund.de/cm/343/bewertung_des_virulenzpotenzials_von shigatoxin_2e*stx2* e_bildenden_e_coli_staemmen.pdf.

[2] Friedrich AW1, Bielaszewska M, Zhang WL, Pulz M, Kuczius T, Ammon A, Karch H. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J Infect Dis. 2002 Jan 1;185(1):74-84. Epub 2001 Dec 14.

[3] Scheiring J1, Andreoli SP, Zimmerhackl LB. Treatment and outcome of Shiga-toxin-associated hemolytic uremic syndrome (HUS). Pediatr Nephrol. 2008 Oct;23(10):1749-60. doi: 10.1007/s00467-008-0935-6. Epub 2008 Aug 13.

[4] Friesema I1, van der Zwaluw K, Schuurman T, Kooistra-Smid M, Franz E, van Duynhoven Y, van Pelt W. Emergence of *Escherichia coli* encoding Shiga toxin 2f in human Shiga toxinproducing E. coli (STEC) infections in the Netherlands, January 2008 to December 2011. Euro Surveill. 2014 May 1;19(17):26-32.

6. Product Description

The RealStar® EHEC PCR Kit 2.0 is a qualitative *in vitro* diagnostic test, 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 a 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.

6.1 Real-Time PCR Instruments

The RealStar® EHEC 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)
- 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.

NOTE	
	***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***

8. Procedure

8.1 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. The suitability of the nucleic acid extraction procedure for use with RealStar® EHEC 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® 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 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® EHEC 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	None

9.2 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)

9.3 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

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® EHEC 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™	ROX™	JOE™
Positive Control [*stx1* + *stx2*+ *ipaH*]	+	+	+	+/-
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™	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.

11. Performance Evaluation

The analytical performance evaluation of the RealStar® EHEC PCR Kit 2.0 was done using quantified *stx1* specific, *stx2* specific and *ipaH* specific DNA.

11.1 Analytical Sensitivity

The analytical sensitivity (limit of detection: LoD) of the RealStar® EHEC PCR Kit 2.0 is defined as the concentration of *stx1*, *stx2* and/or *ipaH* specific DNA that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified *stx1* specific, *stx2* specific and *ipaH* specific DNA.

Table 1: PCR results used for the calculation of the analytical sensitivity with respect to the detection of *stx1* specific DNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
10.000	36	36	100
3.162	36	36	100
1.5	36	36	100
1.000	36	36	100
0.5	36	36	100
0.316	36	31	86
0.100	36	20	56
0.032	36	7	19
0.010	36	4	11
0.003	36	0	0

Table 2: PCR results used for the calculation of the analytical sensitivity with respect to the detection of *stx2* specific DNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
10.000	18	18	100
3.162	18	18	100
1.5	18	18	100
1.000	18	18	100
0.5	18	17	94
0.316	18	17	94
0.100	18	7	39
0.032	18	4	22
0.010	18	1	16
0.003	18	2	11

Table 3: PCR results used for the calculation of the analytical sensitivity with respect to the detection of *ipaH* specific DNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
10.000	18	18	100
3.162	18	18	100
1.5	18	18	100
1.000	18	17	94
0.5	18	15	83
0.316	18	12	67
0.100	18	7	39
0.032	18	1	6
0.010	18	0	0
0.003	18	0	0

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

- For the detection of *stx1* specific DNA, the analytical sensitivity is 0.48 copies/µl [95% confidence interval (CI): 0.33 copies/µl to 0.77 copies/µl]
- For the detection of *stx2* specific DNA, the analytical sensitivity is 0.69 copies/µl [95% confidence interval (CI): 0.30 copies/µl to 3.92 copies/µl]
- For the detection of *ipaH* specific DNA, the analytical sensitivity is 0.96 copies/µl [95% confidence interval (CI): 0.63 copies/µl to 1.88 copies/µl]

11.2 Analytical Specificity

The analytical specificity of the RealStar® EHEC 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 EHEC genotypes will be detected.

The analytical specificity of the RealStar® EHEC PCR Kit 2.0 was evaluated by testing a panel of genomic DNA and RNA extracted from different gastrointestinal pathogens and commensal flora found in the intestine and stool.

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

- Human adenovirus
- Enterovirus 71
- Norovirus (GI)
- Norovirus (GII)
- Rotavirus
- Sapovirus
- Astrovirus
- Hepatitis A virus
- Hepatitis E virus
- *Campylobacter coli*
- *Campylobacter jejuni*
- *Campylobacter lari*
- *Citrobacter freundii*
- *Clostridium difficile*
- *Entamoeba histolytica*
- *Escherichia coli*

- *Giardia lamblia*
- *Listeria innocua*
- *Listeria ivanovii*
- *Listeria monocytogenes*
- *Listeria seeligeri*
- *Listeria welshimeri*
- *Proteus mirabilis*
- *Proteus vulgaris*
- *Salmonella enteritidis*
- *Salmonella typhimurium*
- *Serratia marcescens*
- *Yersinia enterocolitica*

11.3 Precision

Precision of the RealStar® EHEC 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 the C_t-values of Shiga toxin 1 (*stx1*), Shiga toxin 2 (*stx2*) and invasion plasmid antigen H (*ipaH*) specific DNA and of the Internal Control. Twelve replicates (*stx1*) and six replicates (*stx2*, *ipaH*) per sample and per experiment were analysed.

Table 4: Precision data for the *stx1*, *stx2* and *ipaH* specific DNA

stx1, *stx2* and *ipaH*		Average Threshold Cycles (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	*stx1*	28.48	0.09	0.31
	stx2	28.51	0.13	0.45
	ipaH	29.23	0.12	0.41
Inter-Assay Variability	*stx1*	28.46	0.09	0.30
	stx2	28.54	0.11	0.40
	ipaH	29.15	0.16	0.54
Inter-Lot Variability	*stx1*	28.31	0.20	0.71
	stx2	28.42	0.17	0.60
	ipaH	29.20	0.09	0.32
Total Variability	*stx1*	28.35	0.18	0.63
	stx2	28.47	0.17	0.58
	ipaH	29.15	0.13	0.44

Table 5: Precision data for the detection of the Internal Control

Internal Control	Average Threshold Cycles (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	28.71	0.21	0.73
Inter-Assay Variability	28.46	0.34	1.19
Inter-Lot Variability	27.97	0.81	2.88
Total Variability	28.05	0.67	2.40

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 PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- Potential mutations within the target regions of the *ipaH*, *stx1* and/or *stx2* genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- As with any diagnostic test, results of the RealStar® EHEC 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® EHEC 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).

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The RealStar® EHEC 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.

17. Explanation of Symbols

IVD	*In vitro* diagnostic medical device
LOT	Batch code
CAP	Cap color
REF	Product number
CONT	Content
NUM	Number
COMP	Component
GTIN	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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