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Cat No: BS-STI-T-25 / BS-STI-T-100

Sexually Transmitted Infections RT-qPCR Panel

Package Insert

Table 1. Kit Content

Component	Intended Use	25 Reactions	100 Reactions
2X Prime Script Mix	Optimized ready-to-use mix for RT-qPCR assay	1 x 1000 µL	4 x 1000 µL
STI Oligo Mix 1	Specific nucleic acid amplification and detection: **FAM:** Herpes Simplex Virus-1 **HEX:** *Mycoplasma genitalium* **CY5:** Herpes Simplex Virus-2	1 x 125 µL	1 x 500 µL
STI Oligo Mix 2	**HEX:** Human (Internal Control-IC) **ROX:** *Neisseria gonorrhoeae*	1 x 125 µL	1 x 500 µL
STI Oligo Mix 3	**FAM:** *Treponema pallidum* **HEX:** *Trichomonas vaginalis* **ROX:** *Ureaplasma parvum/ urealyticum*	1 x 125 µL	1 x 500 µL
STI Oligo Mix 4	**FAM:** *Mycoplasma hominis* **HEX:** *Haemophilus ducreyi* **ROX:** *Streptococcus agalactiae* **CY5:** *Gardnerella vaginalis*	1 x 125 µL	1 x 500 µL
PC-STI 1 / PC-STI 2 / PC-STI 3 / PC-STI 4	Positive Control (PC)	1 x 100 µL	1 x 100 µL
NTC	Negative Control (NTC)	1 x 1000 µL	1 x 1000 µL

Table 2. Transport Condition, Storage Condition, and Shelf Life of the Components

Component	Transport Condition	Storage Condition*	Shelf Life
2X Prime Script Mix	(-22) °C – (+8) °C	(-22) °C – (-18) °C	12 Months
Oligo Mix		(-22) °C – (-18) °C	
PC		(-22) °C – (-18) °C before opening, (+2) °C – (+8) °C after first thaw	
NTC		(-22) °C – (-18) °C before opening, (+2) °C – (+8) °C after first thaw	

*Each reagent stored at storage temperature can be used until the expiration date indicated on the tube following the first opening. The kit's expiration date is determined by the expiration date of the reagents.

Table 3. Components Required but Not Included with The Test

Components Required but Not Included with The Test
1. Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS) or/and CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad) Real-Time PCR systems
2. Micropipettes and compatible filtered pipette tips (nuclease-free) suitable for transferring 1-10, 10-100, and 100-1000 µL of liquid
3. A centrifuge or Mini-spin
4. Vortex
5. Reaction tubes and caps/films specific to RT-qPCR instruments and compatible with the reaction volume

Table 4. Intended Use, Test Principle, and Analytical Specifications

Function	Aid to diagnosis	**Sample Type(s)**	Table 5
Analyte	Table 1	**Nucleic Acid Preparation Method(s)**	Table 5
Qualitative/Quantitative	Qualitative	**Validated PCR Instruments**	Table 3
Test Principle	Reverse Transcription and Real-Time PCR (RT-qPCR)	**Inclusivity and Exclusivity**	Validated on the reference strains and the field isolates
Automated/Manual	Manual		
Intended Users	Professional use	**Limit of Detection (LoD)**	Table 5
Target Population	Individuals with the suspected infection	**Sensitivity and Specificity**	98.76% and % 99.28%

Table 5. Collection, Storage, and Transfer of Clinical Specimens / Nucleic Acid Preparation Methods and the Respected LoD Values

Sample Type**	Sample Transfer	Sample Storage	Nucleic Acid Preparation Method	LoD (cp/mL)
Throat swab, vaginal swab, urethral swab, urogenital swab, endocervical swab samples	***vNAT® Transfer Tube*** (Cat. No: BS-NA-513m)	3 months at (+2) °C – (+8) °C 1 year at -20 °C	Nucleic acid preparation is not needed. The samples can be used directly in RT-qPCR.	400-800
Urine samples	Viral Transport Medium (VTM) (CDC SOP#: DSR-052-05, without antibiotics)	3 days at (+2) °C – (+8) °C 1 year at -70 °C	***RINA™ M14 Nucleic Acid Extraction Device*** (Robot Catalog No: RINA-M14-01, Kit Cat. No: RN-NA-101) ***Zybio EXM3000 Nucleic Acid Isolation System*** (Robot Model No: EXM3000, Kit Cat. No: ZFNAE01)	100-200
	Preservative-free sterile tubes/containers			100-200

** Clinical specimens should be collected by a healthcare provider in accordance with national/international clinical specimen collection regulations.

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1. RT-qPCR Application Protocol

Before starting the assay, please consider the following:

1. The kit was validated only for the template nucleic acid volume which is 25% of the total RT-qPCR volume.
2. The kit cannot be used with real-time PCR instruments without periodic maintenance records.
3. The kit for Bio-Rad Real-Time PCR systems has been validated with white reaction tubes specific to these systems. Clear reaction tubes result in 5-10 times lower fluorescence signal in Bio-Rad instruments compared to white reaction tubes. In addition, device-specific reaction tubes should be used in the BMS device. The kit's stated analytical performance can only be achieved using validated tubes.
4. To test for contamination, a negative control reaction containing NTC (Nuclease-free Water) must be set up in each run.

Program the RT-qPCR device as follows and add the reagents into the RT-qPCR tubes, close the tubes, place them into the RT-qPCR instrument and start the run (Table 6)

Table 6. Reaction Setup and Real-Time PCR Program

Reaction Setup		**RT-qPCR Program**				**QR Code for Thermal Protocol and Plate Setup**
		CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad) and ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)***				
Reagent	**Volume/Rxn**	**Step**	**Cycle No.**	**Temperature**	**Duration**	
2X Prime Script Mix	10 µL	**Reverse Transcription**	1 Cycle	52 °C	3 min	
		Pre-Incubation	1 Cycle	95 °C	10 sec	
Oligo Mix	5 µL	**Denaturation**	12 Touchdown Cycles: 1 °C decrement in annealing temperature per cycle	95 °C	1 sec	
		Annealing and Extension		67 °C to 56 °C	15 sec	
Template Nucleic Acid/NTC/PC	5 µL	**Denaturation**	30 Cycles	95 °C	1 sec	
		Annealing and Extension		55 °C	15 sec	
Total Reaction Volume	**20 µL**	**Detection (Reading)**		(FAM-Green)/(HEX-Yellow) (ROX-Orange)/(CY5-Red)		https://www.bioeksen.com.tr/files/L_TD_43P/

WARNING: The RT-qPCR thermal programs (Bio-Rad and BMS) and the plate setup (Bio-Rad) file should be downloaded from the QR code or link above.

2. Interpretation of the Assay Results

All default analysis options (e.g., auto-calculated threshold) in the PCR instrument software should not be changed to calculate Cq values.

The shape of the amplification curves should be examined for all reaction wells returning with Cq values. All the sigmoidal curves above the threshold should be recorded as "positive" and their Cq values should be recorded. Non-sigmoidal curves should be recorded as "negative".

Table 7. Expected Performance of Kit Controls

Control Type	**Purpose**	**Expected Results and Cq Values**	
		IC (HEX)	**Target**
Negative Control	Contamination control during RT-qPCR	**Not detected** (No Cq)	**Not detected** (No Cq)
Positive Control	Reagent integrity	**Detected** (Cq≤30)	**Detected** (Cq≤30)
Internal/Extraction Control	To monitor the integrity of nucleic acid extraction and RT-qPCR from each sample	**Detected** (Cq≤30) If the IC is "**Not detected**", check the target Cq.	If the target is "**Detected**" according to the result interpretation criteria, IC is valid.

If any control does not work as described above, the run is reported as follows:

1. **Contamination:** If Cq≤30 in any NTC test channel.
   Recommended action: Repeat the analysis paying attention to the "Warnings and Limitations" section.
2. **Reagent Problem**: In case a sigmoidal curve with a Cq≤30 cannot be obtained for any of all the samples tested in the run, including the controls.
   Recommended action: If all the tested samples show negative results for the target pathogens and controls, the run is considered invalid. In this scenario, it is essential to conduct testing on the "Positive Control(s)" included in the kit. A negative Positive Control test result suggests a potential "Reagent Problem." If encountered, please reach out to the manufacturer for further assistance.
3. **Invalid:** If the IC (Internal Control) and all other targets are "**Not detected**".
   Recommended action: Sampling isn't successfully done, or there is a problem during the sample transportation. A new sample from the same patient should be collected and tested again.

If all the controls are valid, the results are interpreted as follows:

Table 8. Interpretation of Patient Results

Target	**Internal Control (IC)**	**Result Interpretation**	
Positive (+)	**Positive (+)** or **Negative (-)**	Results are valid Target is detected	If 26<Cq ≤30 "Low Positive" If 16<Cq≤26 "Positive" If Cq≤16 "High Positive"
Negative (-)	**Positive (+)**	Results are valid Target is not detected	

The results generated by the qPCR instruments can be reported manually, as explained earlier, or automatically using the "**Sigmoida**" software. To obtain the "Sigmoida" software installer, please send an email to support@bioeksen.com.tr.

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1. Warnings and Limitations

1. False-negative results may occur if inadequate numbers (lower than the LoD) of organisms are present in the specimen.
2. Mutations within the target regions could affect primer and/or probe binding, resulting in failure to detect the presence of agents.
3. The use of cotton or calcium alginate swabs or swabs with wooden sticks can lead to false negative results since they may contain substances that inactivate some viruses and inhibit PCR.
4. A false-negative result may occur if a specimen is improperly collected, transported, or handled.
5. The clinical specimens shall be collected by a healthcare provider in accordance with the specimen collection guidelines.
6. Test procedures should be performed by personnel trained in the use of the kit.
7. Except for liquid transfers, sample tubes should always be kept closed.
8. Filtered and nuclease-free pipette tips should be used for sample transfer.
9. The components in the kit should not be used together with different lot numbers or chemicals of the same name but from different manufacturers.
10. ***The caps of the reaction tubes must not be opened after the PCR run.*** The PCR tubes should be placed in a bag and thrown away after the bag is tightly closed.
11. The surfaces of the workbenches should be wiped with freshly diluted 10% bleach (0.5% NaClO) at the beginning and end of each day.
12. Disposal of waste must be carried out in accordance with local, state, and federal regulations.

2. Explanation of Symbol

Symbol	Title of Symbol	Symbol	Title of Symbol	Symbol	Title of Symbol
CE	European Conformity CE Mark	LOT	Batch code		Keep away from sunlight
IVD	*In vitro* diagnostic *medical device*	REF	Catalogue number		Protect from heat and radioactive sources
	Manufacturer	NON STERILE	Non-*sterile*		Do not use if package is damaged and consult *instructions for use*
	Use-by date		Consult *instructions for use* or consult electronic *instructions for use*		Keep dry
CONTROL –	Negative control		Caution		Keep upright
CONTROL +	Positive control		Temperature limit	Σ	Contains sufficient for *<n>* tests
CONTROL	Control				

3. Manufacturer and Technical Support

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Notice to User: Please inform us about product-related incidents at "**vigilance@bioeksen.com.tr**" within 24 hours.