For in vitro diagnostic use only.
For professional use in laboratories only.

Cat No: BS-SY-MX24S-100

Respiratory RT-qPCR MX-24S Panel

Package Insert

100 Tests

1. Kit Content

Shelf life: 12 months; refer to the expiration date on the box. Each reagent stored at storage temperature, can be used until the expiration date indicated on it. The expiration date of the kit is determined by the expiration date of the reagents.

Table 1a. Kit content

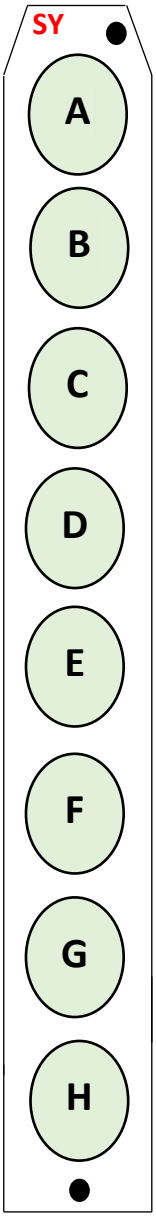
Storage Temperature: -20°C, Transport Temperature: 2-8°C					
Content	**Quantity (100 Test)**	**Content/Intended Use**			
2X Prime Script Mix	140 Tube x 90 µL	DNA polymerase, dNTP mix, reaction buffer, reverse transcriptase, and ribonuclease inhibitor (1 strip for each sample)			
SY Strip	120 Pieces	**Strip**	**Content**	**Channel**	**Target**
			**A**: COVID/Flu Oligo Mix	FAM	SARS-CoV-2 (Nucleocapsid gene)
				HEX	Internal Control (Human *RNase P* gene)
				ROX	Influenza B
				CY5	Influenza A
			B: COR Oligo Mix	FAM	Human Corona 229E
				HEX	Human Corona OC43
				ROX	Human Corona NL63
				CY5	Human Corona HKU1
			C: PAR Oligo Mix	FAM	Parainfluenza 1
				HEX	Parainfluenza 2
				ROX	Parainfluenza 3
				CY5	Parainfluenza 4
			D: MEA Oligo Mix	FAM	Human Metapneumoviruses (MPV)
				HEX	Enterovirus (HEV)
				ROX	-
				CY5	Adenovirus (AV)
			E: BPR Oligo Mix	FAM	Human Bocavirus (HBoV)
				HEX	-
				ROX	Human Parechovirus (HPeV)
				CY5	Rhinovirus (HRV)
			F: LMC Oligo Mix	FAM	*Legionella pneumophila*
				HEX	-
				ROX	*Mycoplasma pneumoniae*
				CY5	*Chlamydophila pneumoniae*
			G: HBS Oligo Mix	FAM	*Haemophilus influenzae*
				HEX	-
				ROX	*Bordetella pertussis*
				CY5	*Streptococcus pneumoniae*
			H: RSV Oligo Mix	FAM	-
				HEX	-
				ROX	Respiratory syncytial virus (RSV) A/B
				CY5	-

Table 1b. Kit content-Controls

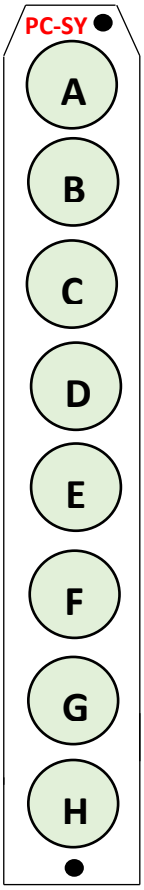
Storage Temperature: -20°C, Transport Temperature: 2-8°C			
NTC (Nuclease Free Water)	2 x 1000 µL	Negative Control **(Test it in each run for contamination control)**	
PC-SY Strip	20 Pieces	**Strip**	**Content (Test it in each run for reaction control)**
			**A**: Positive Control + COVID/Flu Oligo Mix
			B: Positive Control + COR Oligo Mix
			C: Positive Control + PAR Oligo Mix
			D: Positive Control + MEA Oligo Mix
			E: Positive Control + BPR Oligo Mix
			F: Positive Control + LMC Oligo Mix
			G: Positive Control + HBS Oligo Mix
			H: Positive Control + RSV Oligo Mix

Table 1c. Instruments and equipment supplied by the user

Instruments and equipment supplied by the user	
1. Real-Time PCR Instrument: 4 channels, Ramp rate ≥3 °C/sec. **2.** 1-10 µL micropipettes and the compatible filtered tips (DNase and RNase free) **3.** Quick Spin Centrifuge: min. 3000 rpm (compatible with 8 strips and 2 ml microtubes) **4.** Vortex	**Extra components recommended to use:** **5.** UV Cabinet for PCR Setup **6.** Cold Tube Rack (for microcentrifuge tubes and PCR tubes/strips) **7.** Disposable powder-free nitrile gloves

2. Intended Use and Test Principle

The kit is used for the rapid and accurate diagnosis of the viral and bacterial agents given in Table 1a in clinical samples. The kit is applied to nucleic acid isolates obtained from nasopharyngeal swab, oropharyngeal swab, bronchoalveolar lavage, nasopharyngeal aspirate, and sputum samples. Rapid diagnosis with the kit is performed by one-step reverse transcription (RT) and real-time PCR (qPCR) (RT-qPCR), targeting genomic RNA and DNA regions specific to the target agent. With the kit, **diagnosis can be made in less than 45 minutes.**

The "***Bio-Speedy® Respiratory RT-qPCR MX-24S Panel"*** kit is validated with the ***"Bio-Speedy® vNAT® Viral Nucleic Acid Buffer (BS-NA-510)", "Bio-Speedy® vNAT® Transfer Tube (BS-NA-513-100)"*** and ***"RINA™ M14 Nucleic Acid Extraction Robot (RINA-M14-01)"***. The kit is applied to the nasopharyngeal swab, oropharyngeal swab, bronchoalveolar lavage, nasopharyngeal aspirate, and sputum samples taken by healthcare providers from the disease suspected individuals.

3. Analytical Specifications

The kit has been validated for 20 µL qPCR volume using *Bio-Rad CFX96 Touch™* Real Time PCR System and is fully compatible with this system. No cross reaction was observed in analytical specificity studies performed on reference strains and field isolates of the agents given in Table 1a. Limit of Detection (LoD), inclusivity, and exclusivity studies were made by T.R. The Ministry of Health, General Directorate of Public Health, Microbiology Reference Laboratories and Biological Products Department (HSGM). Sensitivity and specificity of the kit was tested on 1152 clinical samples and found 98.95% and 99.13%, respectively. LoD values of the kit determined by HSGM were 66 copies/mL for Influenza A, 88 copies/mL for Influenza B, 67 copies/mL for SARS-CoV-2, 62 copies/mL for Coronavirus 229E, 68 copies/mL for Coronavirus OC43, 94 copies/mL for Coronavirus NL63, 86 copies/mL for Coronavirus HKU1, 75 copies/mL for Parainfluenza 1, 91 copies/mL for Parainfluenza 2 , 53 copies/mL for Parainfluenza 3, 88 copies/mL for Parainfluenza 4, 64 copies/mL for Metapneumovirus, 71 copies/mL for Enterovirus, 62 copies/mL for Adenovirus, 83 copies/mL for Bocavirus, 59 copies/mL for Parechovirus, 97 copies/mL for Rhinovirus, 54 copies/mL for *Legionella pneumophila,*

62 copies/mL for *Mycoplasma pneumonia, 58* copies/mL for *Chlamydophila pneumoniae, 48* copies/mL for *Haemophilus influenzae, 47* copies/mL for *Bordetella pertusis*, 43 copies/mL for Streptococcus pneumoniae, and 97 copies/mL for Respiratory syncytial virus A/B.

The clear reaction tubes result in 5-10 times lower fluorescence signal in the *Bio-Rad CFX 96* instrument when compared to the white tubes. The specified analytical performance of the kit can only be achieved using the validated white tubes.

4. Collection, Storage and Shipment of Clinical Specimens

Swab samples should be collected using Dacron or Polyester swabs. Other specimen types should be transferred in sterile containers. In the transport phase, Viral Transport Medium (VTM) (Preparation of viral transport medium, Center for Disease Control and Prevention, SOP#: DSR-052-01) or ***"Bio-Speedy® vNAT® Transfer Tube (BS-NA-513-100)"*** should be used. Samples should be stored and transported at 2-8°C until they arrive at the laboratory. Swab samples should be transferred within 5 days, other sample types should be transferred within 2 days. If a delay in shipment is expected, samples should be frozen at -70°C and shipped with dry ice. It is important that samples should not be exposed to the repeated freeze-thaw.

5. Warnings

1. Store the kit away from nucleic acid sources and qPCR amplicons.
2. Do not mix the kit components with different lot numbers or chemicals of the same name but from different manufacturers.
3. Keep the master stock reagents on the cold block during the PCR setup.
4. If it is possible, setup PCR on the cold block.
5. Mix the kit components gently before use.
6. Use separate micropipettes for pipetting qPCR mixes and template nucleic acids.
7. Always keep the template nucleic acid and positive control tubes closed, except for the fluid transfers.
8. Regularly clean the wipeable surfaces of the rooms, benches, and devices where the test is performed with 10% NaClO.
9. Disposed of the qPCR completed reaction tubes before opening in the laboratory.

6. RT-qPCR Application Protocol

Perform the reaction setup as indicated in Table 2 and program the qPCR instrument according to Table 3. Cap the qPCR tubes, place them in the qPCR instrument and start the run.

Table 2. Reaction setup

Reaction Type	Strip to use	1 x 2X Prime Script Mix tube is added in the following amounts of sample, mixed with pipetting, distributed to the strip wells in the amount given in the right column.		Amount to be distributed to A, B, C, D, E, F, G and H wells
Patient Sample	**SY** 1 strip for each patient	2X Prime Script Mix	90 µL	15 µL
		Patient sample nucleic acid extract	45 µL	
		Total	135 µL	
Negative Control (NTC)	**SY** 1 strip for each run	2X Prime Script Mix	90 µL	15 µL
		NTC	45 µL	
		Total	135 µL	
Positive Control (PC)	**PC-SY** 1 strip for each run	2X Prime Script Mix	90 µL	10 µL

Table 3. Real-Time PCR Program; Set the reaction volume to 20 µL

Reverse Transcription/1 Cycle	Pre-Incubation/1 Cycle	Amplification/40 cycle		
		Denaturation	Annealing and Extension	Fluorescent Reading
52°C-5 min	95°C-10 sec	95°C-1 sec	55°C-10 sec	FAM/HEX/ROX/CY5

7. Interpretation of the Assay Results

The recommended threshold level to calculate the number of threshold cycles (Cq) is 200 RFU for *CFX96 Touch™* instruments.

The shape of the amplification curves should be examined. If a Cq value is assigned to a sample by the instruments' software and the curve is sigmoidal, the Cq value can be used in the final evaluation. **Non-sigmoidal curves should be recorded as negative.** If a Cq value is assigned to a sample, but the curve is not sigmoidal, **the result should be recorded as negative.**

For samples with a suspected sigmoidal curve pattern under the threshold in targets' channel, Cq value of the IC should be examined. If the IC Cq≤34, the sample is reported as negative. If the Cq>34, the test should be repeated after freezing and thawing the sample. If the problem continues after the freezing and thawing, a new sample is requested.

WARNING: On the web page linked with the QR code, examples of the sigmoidal amplification curves are given. The results obtained with this kit should NOT be interpreted without examining these samples.

Table 4. Expected performance of the kit controls

Control Type	Name	Control purpose	Expected Result	
NTC addition	NTC	Contamination control	No Cq = Valid	
No template addition	NRC	Reactive contamination control	No Cq = Valid	
PC addition	PC	Positive reactive control	Cq≤38.0 = Valid	
Human mRNA*	IC	Control of the sampling, RNA integrity, nucleic acid extraction, inhibition of both reverse transcription and qPCR	Cq≤34.0 = Valid	If IC Cq>34.0, and if target Cq≤38.0, conclude as IC is valid
*** If any target is positive in a multiplex reaction with internal control, the target is interpreted as positive even if the internal control (IC) is negative. If any target is not positive in the multiplex reaction with internal control, the internal control (IC) should give a positive result.**				

If any control does not perform as described above, the run is considered invalid, and the test is repeated.

1. Invalid PC: Contact the manufacturer, renew the reagents, and repeat the reaction.
2. Invalid NRC: Contact the manufacturer, renew the reagents, and repeat the reaction.
3. Invalid NTC: Repeat the analysis by paying attention to the "**Warnings**" section.
4. Invalid IC: Repeat the analysis. If the problem continues, then conclude as an invalid PCR template.

If all the controls are valid, proceed to the interpretation of the results.

- If Cq of the gene targets are **≤38**, conclude as **positive**.
- If Cq of the gene targets are **>38**, conclude as **negative**.

8. Limitations

- Performance of the *Bio-Speedy® Respiratory RT-qPCR MX-24S Panel* has only been established in nasopharyngeal swab, oropharyngeal swab, bronchoalveolar lavage, nasopharyngeal aspirate, and sputum samples.
- Mutations within the target regions of the *Kit* could affect primer and/or probe binding resulting in failure to detect the presence of virus or bacteria.
- A false negative result may occur if a specimen is improperly collected, transported, or handled.
- Inhibitors or other types of interference may produce a false negative result. False negative results may also occur if inadequate numbers of organisms are present in the specimen.

9. Explanation of Symbol

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
CE	European Economic Area	IVD	For In Vitro Diagnostic Use		Keep away from light
	Manufacturer	LOT	Batch code		Protect from heat and radioactive sources
	Use-by Date (Expiration Date) YYYY-MM	REF	Catalog Number		Do not use if package is damaged and consult instructions for use
CONTROL -	Negative Control	NON STERILE	Non-Sterile		Keep away from water/moisture
CONTROL +	Positive Control		Consult Instructions for Use		Fragile, handle with care
	Temperature limit (Store temperature)		Caution		Keep it upright

10. 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.