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INSTRUCTION MANUAL

Quick SARS-CoV-2 Multiplex Kit

Catalog No. **R3013, R3013-1K, R3013-10K**

Highlights

- **High Sensitivity:** Limit of Detection as low as 5 viral genome equivalent copies/reaction (165 GEC/ml).
- **Rapid & Easy Setup:** Ready-to-use one-step master mix. Results in less than 2 hours after extraction.
- **Flexible**: Compatible with Manual, Automated, and High-Throughput workflows.
- **Extremely Accurate:** 100% agreement with expected results in negative and contrived positive samples.

Contents

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Product Contents

Quick SARS-CoV-2 Multiplex Kit (Kit Size)	R3013 (100 rxn**)	Storage Temperature
2X CV Mix*	1 ml	≤ -70°C
CV Positive Control*	100 µl	≤ -70°C
No Template Control (NTC)	3 x 1 ml	Room Temp.

Note - Integrity of kit components is guaranteed for up to the claimed expiration date on the kit under proper storage conditions. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

*Reagents are stable for up to five (5) freeze-thaw cycles.
**Kits are also available in 1,000 reaction (R3013-1K) and 10,000 reaction (R3013-10K, bulk reagent) format.

Specifications:

- **Sample Input Material:** Extracted RNA from different sources (e.g. swabs, sputum, respiratory fluids).
- **Input Quality:** Extracted RNA free of enzymatic inhibitors.
- **Equipment Required:** Real-Time PCR Instruments capable of detecting HEX™ (or VIC®) and Quasar® 670 (or Cy5®) fluorophores.
- **Reagents:** Ready-to-use master mixes and controls.
- **Processing Time:** Results in < 2 hours after extraction.
- **Compatibility:** Compatible with manual, automated, and high-throughput workflows.

Required Equipment and Consumables Not Provided

- Microcentrifuge
- Vortex
- Microplate Centrifuge
- Pipettes and aerosol barrier filter tips.
- 96-well PCR plates
- Optically clear film for Real-Time PCR
- CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad) capable of detecting **HEX™** and **Quasar® 670**, or other Real-Time PCR detection systems equipped with an optic component compatible with HEX™ and Quasar® 670 or fluorophores with similar excitation and emission wavelengths.

This product is for research use only and should only be used by trained professionals. Follow the safety guidelines and rules enacted by your research institution or facility.

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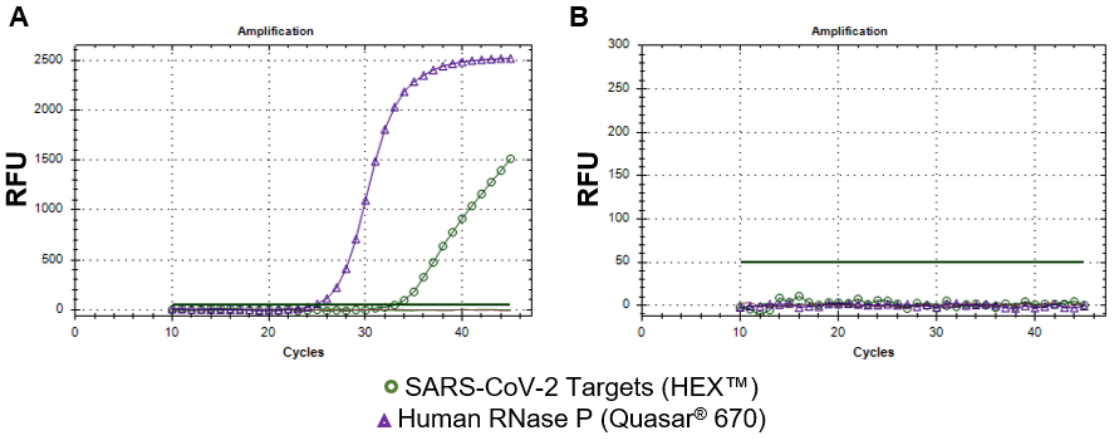
Product Description

The ***Quick* SARS-CoV-2 Multiplex Kit** is a real-time reverse transcription PCR (rRT-PCR) test for the qualitative detection of RNA from the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which is responsible for COVID-19. The *Quick* SARS-CoV-2 Multiplex Kit detects one SARS-CoV-2 specific target from the nucleocapsid (N) gene and one host-specific target from the human RNase P gene.

The **2X CV Mix** already contains the primers and probes necessary to detect the SARS-CoV-2 nucleocapsid gene targets (labeled with the HEX™ fluorophore) as well as the human RNase P (RP) target, which is used to assess sample and extraction quality. The RP probe is labeled with the Quasar® 670 fluorophore.

The kit also includes a **CV Positive Control** and a **No Template Control (NTC)** that enable monitoring for failures or contamination of the reagents and reaction conditions. The CV Positive Control contains *in vitro* transcribed fragments of the SARS-CoV-2 N gene as well as human RNA. The NTC contains DNase/RNase-free water. Typical amplification curves for the CV Positive Control **(A)** and NTC **(B)** are illustrated below:



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Notes:

[1] High RNA quality is a key factor in guaranteeing optimal performance of the assay. We recommend extracting RNA using *Quick*-DNA/RNA Viral MagBead, Quick-DNA/RNA Viral Kit, or Quick-RNA Viral Kit (Cat. no. R2140, D7020, R1034, Zymo Research), or similar, to obtain high-quality RNA from samples.

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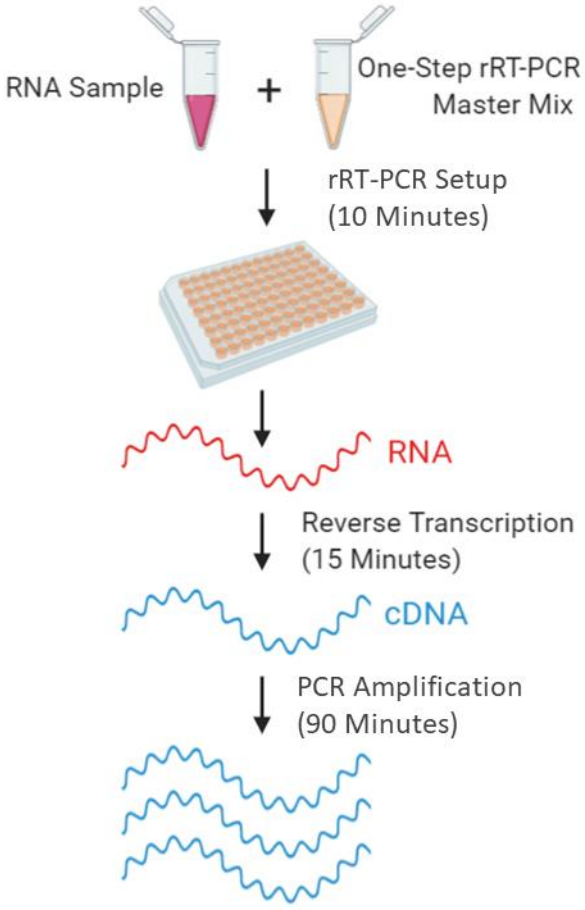
Procedure Overview:

This assay can be used with RNA[1] extracted from a variety of sources, including respiratory fluids and nasal or throat swabs.

As illustrated in the workflow figure below, RNA is mixed with 2X CV Mix followed by incubation in a Real-Time PCR System. Results are ready to be analyzed after the reverse transcription and real-time PCR steps are completed.



Warnings, Precautions, and Experimental Considerations:

- This product is for research use only.

- Handle all specimens as if infectious using safe laboratory procedures.

- As with all PCR-based assays, *Quick* SARS-CoV-2 Multiplex reagents are sensitive to accidental contamination with sources of target-specific nucleic acids, in particular, PCR products from previous amplification reactions and SARS-CoV-2 positive samples.
  - Never open used rRT-PCR plates.
  - If possible, set up the rRT-PCR plates in an area separate from sample processing and RNA extraction.
  - Aliquot 2X CV Mix in the 96-well PCR plate before handling any test samples or included controls.
  - Follow good laboratory practice in order to minimize the risk of cross-contamination during rRT-PCR set up.
  - Decontaminate work surfaces, pipettes, and centrifiges before and after the set-up of the rRT-PCR plates.
  - Always use aerosol barrier filter tips for sample handling and during rRT-PCR set-up.

- *Quick* SARS-CoV-2 Multiplex Kit components are temperature sensitive.
  - With the exception of the NTC, all *Quick* SARS-CoV-2 Multiplex Kit components must be thawed on ice prior to use and stored at ≤ -70°C when not in use.
  - While setting up the assay, keep all *Quick* SARS-CoV-2 Multiplex Kit components, with the exception of the NTC, and RNA samples on ice to ensure optimal performance.
  - PCR plates should be kept on ice during reaction set-up and until loaded into the Real Time PCR instrument.
  - *Quick* SARS-CoV-2 Multiplex Kit components are stable for up to five (5) free-thaw cycles.

- Protect the 2X CV Mix and 96-well PCR plate from light when preparing the reactions.

- Do not use expired reagents.

Notes:

Procedure:

I. rRT-PCR Reaction Set Up:

Before starting, thaw frozen reagents on ice, mix 10 times by inversion, centrifuge briefly, and place back on ice.

Avoid exposing the 2X CV Mix and reactions to direct light and keep the 96-well PCR plate on ice during preparation.

To prevent contamination, handle all reagents carefully and aliquot the 2X CV Mix in the 96-well PCR plate before handling any test samples or controls.

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Notes:

1. Add 10 µl of **2X CV Mix** to each well.
2. Add 10 µl of every RNA sample to be tested.
3. Add 10 µl of **CV Positive Control** to one dedicated well in each plate.
4. Add 10 µl of extracted **No Template Control (NTC)** to one dedicated well in each plate.

Example plate layout for a Quick SARS-CoV-2 Multiplex assay is illustrated below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	NTC
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	PC

S# = Test Sample
NTC = No Template Control
PC = CV Positive Control

5. Firmly seal the 96-well PCR plate with an optically transparent sealing film.
6. Briefly vortex the 96-well PCR plate and centrifuge to eliminate bubbles and bring any droplets to the bottom of the well.
7. Place the 96-well PCR plate on ice.

II. Real-Time PCR Machine Set Up:

1. Using the Real-Time PCR software (e.g. Bio-Rad CFX Maestro for the CFX96 Touch™ Real-Time PCR Detection System), create the following PCR program using the following parameters:

Step	Temperature	Time (min:sec)	
1	55°C	15:00	
2	95°C	10:00	
3	95°C	0:05	45 Cycles
4	72°C	0:30	
5	57°C	0:30	
6	**Plate Read**		
7	**END**		

2. Assign each well the corresponding sample names, targets, and fluorophores. **2X CV Mix** contains 2 different fluorophores:

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Targets	Fluorophores	Ex.	Em.
N1	HEX™	538 nm	555 nm
RP	Quasar® 670	647 nm	666 nm

3. Enable all the light filters required to detect both fluorophores.

4. Load the 96-well PCR plate into the Real-Time PCR machine and start the program.

III. Data Analysis (for Bio-Rad CFX Maestro™ software):

1. Visually inspect the plate for any issues that occurred during PCR (e.g. evaporation due to improper plate sealing) and take note of any problems.

2. Adjust the Fluorescence Baseline Threshold Value to 50 RFU for each fluorophore. Under **"Settings**", click on **"Baseline Threshold"**, select **"User Defined"** and enter 50 into the user defined field.

3. Under **"Settings**", click on **"Baseline Settings"**, and select **"Apply Fluorescence Drift Correction"**.

4. Note and record any sample with amplification in the HEX™ or Quasar® 670 channel before 10 cycles. This step is necessary to identify samples with Ct values < 10 which may be excluded by adjustments made in **Step 5**.

5. Exclude the first 10 cycles of the PCR by going to **"Settings"**, clicking on **"Cycles to Analyze."** Manually enter the range as 10 to 45.

6. Visually inspect the signals for the controls and verify their validity using the information provided in the **Interpretation of Results** section on **page 7**.

7. Visually inspect the amplification curves for RNase P target and note any sample with no RNase P amplification or RNase P signal greater or equal to cycle 40.

8. Visually inspect one-by-one[1] the amplification curve for each sample that exhibits SARS-CoV-2 signal. Note any sample displaying aberrant amplification curves. Please refer to the **Appendix** on **page 8** for examples and detailed explanations of aberrant amplification curves.

Notes:

[1]It is important to inspect each sample with SARS-CoV-2 signal individually. The presence of signal from other samples may automatically adjust the scale of the Y axis to a higher RFU making it impossible to analyze the curve of samples with late and/or weak SARS-CoV-2 signal in detail.

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Interpretation of Results:

Guidelines for interpreting results written in this section are provided as a suggestion and are based upon results obtained using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad).

Ct values provided in this section are representative and may vary between different Real-Time PCR instruments.

All controls should be examined prior to the interpretation of the test samples:

CV Positive Control: The Positive Control sample will show amplification signals in both the HEX™ and Quasar® 670 channels, which detect the presence of SARS-CoV-2 and RNase P, respectively. Signals generated from the Positive Control will be considered valid if the cycle threshold (Ct) value is $\leqslant$ 40 for the virus (N) targets (HEX™ channel) and $\leqslant$ 30 for RNase P (Quasar® 670 channel) when using the recommended systems settings. If signals for the Positive Control are detected after 40 amplification cycles (Ct > 40) for the virus targets or after 30 amplification cycles (Ct > 30) for RNase P, the control must be replaced. If this problem is not resolved, the whole kit must be replaced with a new one.

No Template Control: This control should not produce any PCR signal for either the RNase P target (Quasar® 670) or the SARS-CoV-2 targets (HEX™). Amplification in either channel potentially indicates cross-contamination problems (during the sample extraction or during the RT-PCR assembly), or accidental contamination of the rRT-PCR reagents; the run must be repeated and/or reagents must be replaced

Use the following table to interpret the results of all test samples if the controls performed as expected:

Input	RNase P Target (Quasar® 670)	SARS-CoV-2 N1 Targets (HEX™)	Interpretation
CV Positive Control	Ct ≤ 30	Ct ≤ 40	Valid
No Template Control	No Amplification	No Amplification	Valid
Test Sample	Any or no amplification	CT ≤ 40	Positive
	Ct < 40	No amplification or Ct > 40	Negative
	No amplification or Ct ≥ 40	No amplification	Invalid*

* If the sample is repeatedly invalid a new sample should be obtained.

Notes:

Quick SARS-CoV-2 rRT-PCR Kit test results should be used for research use only and not for diagnostic purposes.

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Please consult the **Troubleshooting Guide** on **page 10** if results deviate from these guidelines.

Appendix

Examples of Normal and Aberrant Amplification Curves:

Please note that as indicated in section III of the protocol on **page 6**, the first 10 cycles of PCR have been excluded from the graphs and the baseline threshold for both fluorophores has been adjusted to 50 RFU.

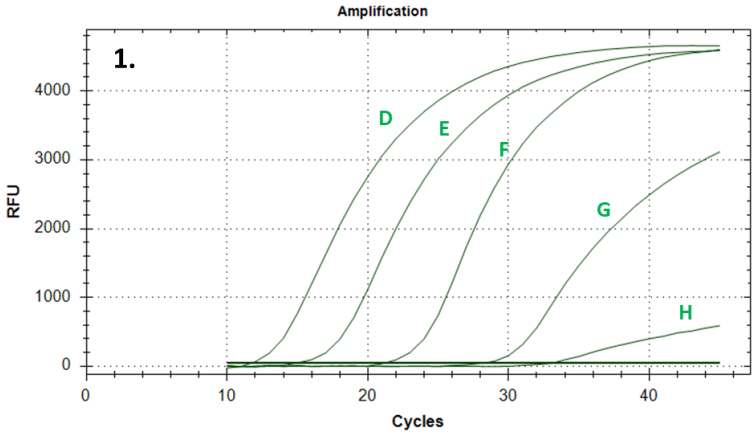

Figure 1. Curves D, E, F, G, and H (Green Letters) are examples of normal amplifications for SARS-CoV-2 targets. H represent the SARS-CoV-2 signal in the CV Positive Control.

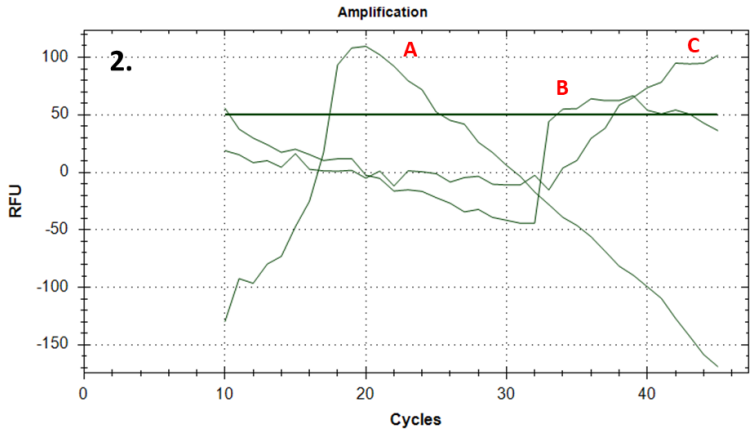

Figure 2. Curves A, B, and C (Red Letters) are examples of aberrant amplifications for the SARS-CoV-2 targets. The Y axis scale has been adjusted (magnified) to visualize details of the curves.

Notes:

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Notes:

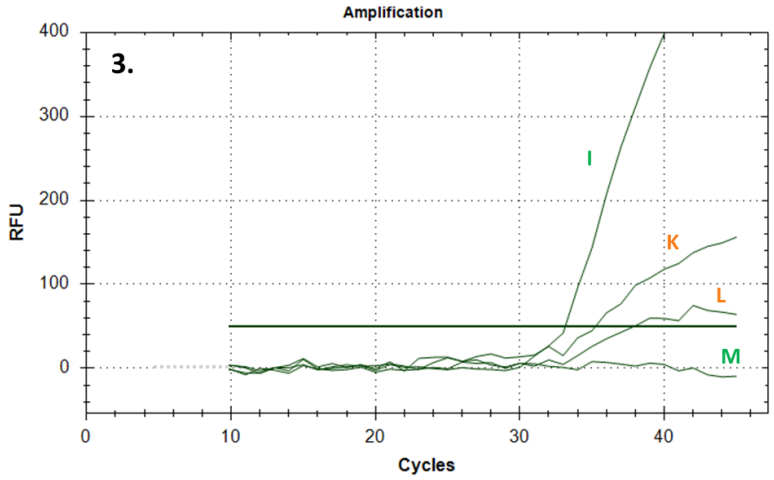

Figure 3. Curve I is considered a positive signal for SARS-CoV-2 target. Curves K and L can be either weak positive or aberrant signals for the SARS-CoV-2 target. For these two cases, the rRT-PCR reaction should be repeated to confirm the results. Curve M is a negative signal. The scale of the Y axis has been adjusted to 400 RFU to better visualize the differences between the curves.

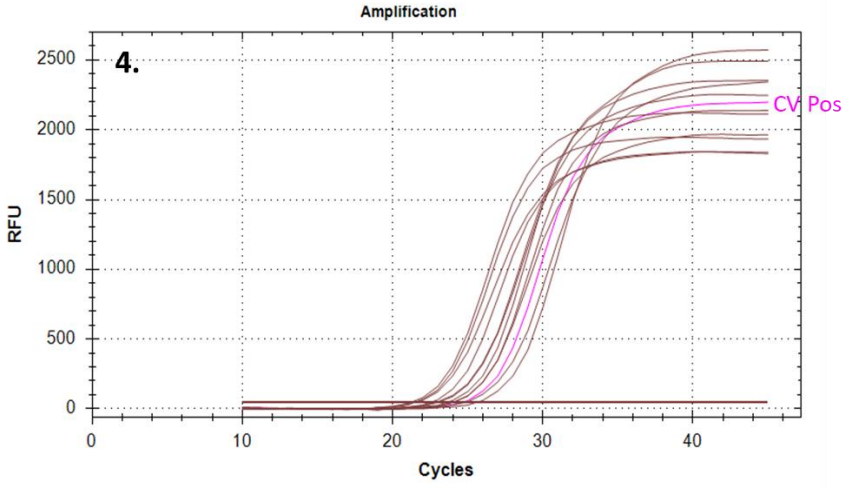

Figure 4. Examples of samples with normal amplification curves for RNase P are shown in brown. Signals for a SARS-CoV-2 positive control is indicated in pink.

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Troubleshooting Guide:

Problem	Possible Causes and Suggested Actions
Reduction in volume observed in the wells after rRT-PCR.	• **Cause:** The PCR reaction evaporated because the PCR plate was not sealed properly. • **Action:** Record the wells with reduced volume and proceed with normal analysis. Repeat rRT-PCR on samples experiencing evaporation if results are invalid.
Aberrant amplification after Data Analysis settings have been adjusted as on page 6. Please see examples of aberrant and normal amplification curves in the Appendix on page 8.	• **Cause:** Presence of air bubbles in the reaction, poor quality sample, incorrect set up, or compromised reagents. • **Action:** Record the wells and fluorophores that show aberrant amplification. Samples that exhibit aberrant amplification should be repeated to determine a conclusive result. Re-extraction of the sample or use of new reagents may be necessary if re-testing the sample does not produce a clear signal.
SARS-Cov-2 signal in CV Positive Control detected after 40 Cycles.	• **Cause:** Incorrect Set-Up, or the *Quick* SARS-CoV-2 Multiplex Kit reagents may have been compromised (e.g. improper storage, or more than 5 freeze-thaw cycles). • **Action:** Re-run the test. If the problem persists, the entire kit should be replaced.
RNase P signal in CV Positive detected after 30 Cycles.	• **Cause:** Incorrect Set-Up, or the *Quick* SARS-CoV-2 reagents may have been compromised (e.g. improper storage, or more than 5 freeze-thaw cycles). • **Action:** Re-run the test. If the problem persists, the entire kit should be replaced.
SARS-CoV-2 and/or RNase P amplification in the No Template Control (NTC)	• **Cause:** Contamination from the environment, contamination of extraction and/or rRT-PCR reagents, or well-to-well cross contamination. • **Action:** Decontaminate all surfaces and instruments with sodium hypochlorite or ethanol. Ensure that filters tips are used during the procedure and changed between samples. Ensure that extraction and rRT-PCR set-up are properly executed. Repeat extraction and rRT-PCR with new reagents including all required controls.

Ordering Information:

Product Description	Kit Size	Catalog No.
Quick **SARS-CoV-2 Multiplex Kit™**	100 rxn 1,000 rxn 10,000 rxn	R3013 R3013-1K R3013-10K

Related Products:

For Sample Collection	Amount	Catalog No.
DNA/RNA Shield™	50 ml 250 ml	R1100-50 R1100-250
DNA/RNA Shield™ (2x Concentrate)	25 ml 125 ml	R1200-25 R1200-125
DNA/RNA Shield™ Collection Tube w/swab (1 ml fill)	10 Pack 50 Pack	R1106 R1107
DNA/RNA Shield™ Collection Tube w/swab (2 ml fill)	10 Pack 50 Pack	R1108 R1109
DNA/RNA Shield™ Saliva Collection Kit	1 x 2ml fill	R1210

For Viral RNA Extraction	Kit Size	Catalog No.
Quick-RNA™ Viral Kit	50 preps 200 preps	R1034 R1035
Quick-RNA™ Viral 96 Kit	2 x 96 preps 4 x 96 preps	R1040 R1041
Quick-DNA/RNA™ Viral kit	50 preps 200 preps	D7020 D7021
Quick-DNA/RNA™ Viral MagBead	1 x 96 preps 4 x 96 preps	R2140 R2141
Quick-DNA/RNA™ Viral 96 Kit	2 x 96 preps 4 x 96 preps	D7022 D7023

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