

MagMAX Viral/Pathogen kit protocol changes to enable increased SARS-CoV-2 testing throughput

Reduction in sample input volume and wash steps, plus alternative KingFisher plastics

This application note provides performance data on the Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit and MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit, and guidance on the following technical elements for SARS-CoV-2 testing:

- Reduction of sample input volume from 400 μL to 200 μL
- Reduction from 3 wash steps to 2 wash steps
- Use of alternative plastic plates and tip combs for Thermo Scientific™ KingFisher™ instruments

Equivalent performance of MagMAX Viral/Pathogen and MagMAX Viral/Pathogen II Nucleic Acid Isolation Kits

The MagMAX Viral/Pathogen kit and MagMAX Viral/Pathogen II kit demonstrate equivalent performance as measured by C_t values of spiked SARS-CoV-2 RNA, enabling greater flexibility and testing capacity (Figure 1). The protocols for the MagMAX Viral/Pathogen kit and MagMAX Viral/Pathogen II kit result in equivalent RNA purification efficiency whether using 400 μL or 200 μL of sample input (and normalized going into the qRT-PCR reaction). The component volumes per well and C_t values are shown in Table 1 and Figure 1, respectively. Samples were processed on a Thermo Scientific™ KingFisher™ Flex Purification System with 96 Deep-Well Head.

Table 1. Sample prep protocol for MagMAX Viral/Pathogen kits.

	400 μL sample input	200 μL sample input
Component	Volume per well	
Proteinase K	10 μL	5 μL
Sample (or nuclease-free water for negative control)	400 μL	200 μL
Binding bead mix (binding solution + beads)	550 μL (530 μL + 20 μL)	275 μL (265 μL + 10 μL)
MS2 phage control	10 μL	5 μL

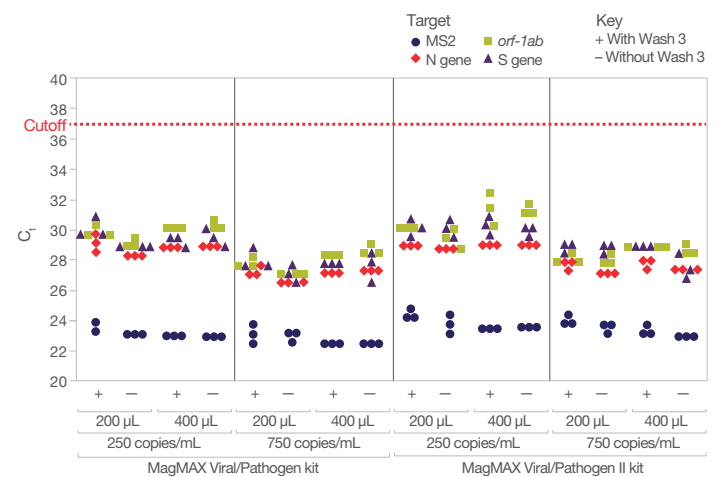


Figure 1. C_t values for contrived SARS-CoV-2-positive nasopharyngeal swab samples with different sample preparation workflows. Nasopharyngeal swab specimens were spiked with 250 copies/mL or 750 copies/mL of SARS-CoV-2 viral RNA (1x or 3x the limit of detection, respectively), and RNA was extracted from 200 μL or 400 μL of the spiked sample in triplicate using either the MagMAX Viral/Pathogen or MagMAX Viral/Pathogen II kit with or without Wash 3. After elution in 50 μL , the samples were assayed by qRT-PCR. All negative samples had C_t values of “Undetected” (data not shown).

Reduction from 3 wash steps to 2 wash steps

The MagMAX Viral/Pathogen kit and MagMAX Viral/Pathogen II kit protocols for SARS-CoV-2 testing using the KingFisher Flex Purification System with 96 Deep-Well Head support both 3 wash steps (Tables 2 and 3) and 2 wash steps (Tables 4 and 5).

Table 2. Protocol for 400 µL sample input with 3 wash steps (script name: MVP_Flex_400ul.bdz).

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 plate	2	KingFisher deep-well 96 plate	Wash buffer	1,000 µL
Wash 2 plate	3		80% ethanol	1,000 µL
Wash 3 plate	4		80% ethanol	500 µL
Elution plate	5		Elution solution	50 µL
Tip comb	6	Place a KingFisher 96 tip comb for deep-well magnets in a KingFisher 96 microplate or alternative tip comb-presenting plate		

Table 3. Protocol for 200 µL sample input with 3 wash steps (script name: MVP_Flex_200ul.bdz).

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 plate	2	KingFisher deep-well 96 plate	Wash buffer	500 µL
Wash 2 plate	3		80% ethanol	500 µL
Wash 3 plate	4		80% ethanol	250 µL
Elution plate	5		Elution solution	50 µL
Tip comb	6	Place a KingFisher 96 tip comb for deep-well magnets in a KingFisher 96 microplate or alternative tip comb-presenting plate		

Updated scripts may be obtained at thermofisher.com/magmaxviralpathogen, or by editing the existing scripts according to Tables 4 and 5.

Table 4. Protocol for 400 µL sample input with 2 wash steps (script name: MVP_2Wash_400_Flex.bdz).

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 plate	2	KingFisher deep-well 96 plate	Wash Buffer	1,000 µL
Wash 2 plate	3		80% ethanol	1,000 µL
Elution plate	4		Elution solution	50 µL
Tip comb	5	Place a KingFisher 96 tip comb for deep-well magnets in a KingFisher 96 microplate or alternative tip comb-presenting plate		

Table 5. Protocol for 200 µL sample input with 2 wash steps (script name: MVP_2Wash_200_Flex.bdz).

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 plate	2	KingFisher deep-well 96 plate	Wash buffer	500 µL
Wash 2 plate	3		80% ethanol	1,000 µL
Elution plate	4		Elution solution	50 µL
Tip comb	5	Place a KingFisher 96 tip comb for deep-well magnets in a KingFisher 96 microplate or alternative tip comb-presenting plate		

Here we show the reduction from 3 wash steps to 2 wash steps without any loss of sensitivity for the MagMAX Viral/Pathogen kit and the MagMAX Viral/Pathogen II kit (Figure 1). This 2-wash protocol conserves both processing plastics and wash reagents as well as time and effort. This protocol has been tested with qRT-PCR; other applications have not been evaluated.

Additional advantages of reducing wash steps

Reduction from 3 wash steps to 2 wash steps in both the 400 and 200 uL protocols, not only reduces the amount of plastics needed by 20% but also decreases the amount of 80 % ethanol solution required for washing by 24 mL - 48 mL per plate depending on whether the 200 uL or 400 uL input volume is used.

SKU	Description	Quantity	No. boxes required / 9600 samples 3 wash protocol	No. boxes required / 9600 samples 2 wash protocol
97002534	KingFisher 96 tip comb for deep well magnets	100/box	1	1
95040450	KingFisher 96 deep-well plates	50/box	10	8
97002540	KingFisher 96 (200 uL) microplate	48/box	3	3

Ordering information

Product	Quantity	Cat. No.
Recommended MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit	Up to 2,000 preps	A48383
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	Up to 200 preps	A42352
KingFisher Flex Purification System with 96 Deep-Well Head	1 system	5400630
KingFisher 96 deep-well plate, V-bottom, polypropylene	50/box	95040450
KingFisher 96 (200 µL) microplate	48/box	97002540
KingFisher 96 tip comb for deep-well magnets	100/box	97002534

Find out more about MagMAX Viral/Pathogen Nucleic Acid Isolation Kits at thermofisher.com/magmaxviralpathogen

Find out more about KingFisher plastics at thermofisher.com/kingfisherplastics

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