

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 μ L to 200 μ L
- 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 (Cat. No. A48310) and MagMAX Viral/Pathogen II kit (Cat. No. A48383) 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 μ L or 200 μ L 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 (Cat. No. 5400630).

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

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

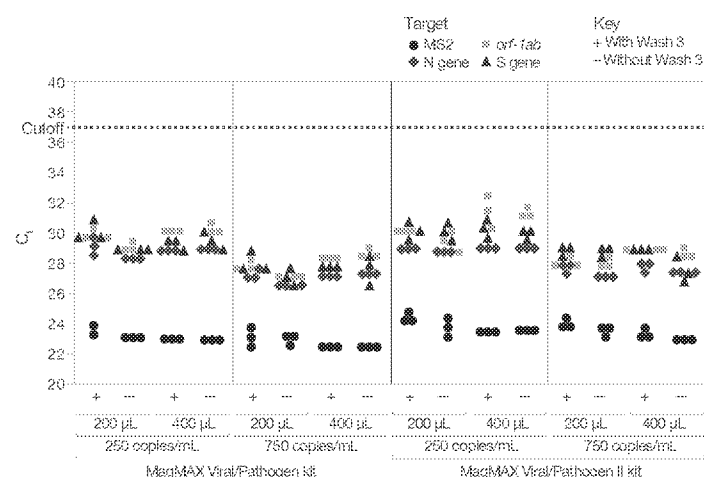


Figure 1. C_t values for contrived SARS-CoV-2-positive nasopharyngeal swab samples with different sample preparation workflows. Nasopharyngeal swab specimens were spiked with 250 copies/mL or 750 copies/mL of SARS-CoV-2 viral RNA (1x or 3x the limit of detection, respectively), and RNA was extracted from 200 μ L or 400 μ L 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 μ L, 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.

Alternative plastics for KingFisher instruments

The following products can be used as alternatives for the existing Thermo Scientific™ KingFisher™ 96 deep-well, V-bottom plate (Cat. No. 95040450).

Plate description	KingFisher instrument compatibility	Quantity	Cat. No.
New KingFisher 96 deep-well plate	Flex, Duo Prime	50 plates per case	95040455
New KingFisher 96 deep-well plate			A48424*
New KingFisher 96 deep-well plate	Flex, Duo Prime, Presto		A48305

The following products can be used as alternatives for the existing Thermo Scientific™ KingFisher™ 96 tip comb for deep-well magnets (Cat. No. 97002534).

Tip comb description	KingFisher instrument compatibility	Quantity	Cat. No.
New KingFisher 96 tip comb for deep-well plates	Flex, Presto	100 tip combs per case	A48438
New KingFisher 96 tip comb for deep-well plates		50 tip combs per case	A48414*

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 2,000 preps	A48310
KingFisher Flex Purification System with 96 Deep-Well Head	1 system	5400630
KingFisher 96 deep-well plate, V-bottom, polypropylene	50/box	95040450
New KingFisher 96 deep-well plate, V-bottom, polypropylene	50/box	A48305
New KingFisher 96 deep-well plate (for Flex and Duo Prime instruments only)	50/box	95040455
New KingFisher 96 deep-well plate (for Flex and Duo Prime instruments only)	50/box	A48424*
New KingFisher 96 tip comb for deep-well plates	100/box	A48438
New KingFisher 96 tip comb for deep-well plates	50/box	A48414*
KingFisher 96 (200 µL) microplate	48/box	97002540
KingFisher 96 tip comb for deep-well magnets	100/box	97002534

* Not available in all regions.

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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