

Influenza SARS-CoV-2 assay for Countable PCR

DESCRIPTION

Why this assay matters

The SARS-CoV-2 *NI*, Influenza A *M1*, Influenza B *NS2*, and *RPP30* targets are key biomarkers for respiratory virus research. Sensitive, reproducible detection enables high-confidence biomarker assessment with every run.

The Countable Influenza SARS-CoV-2 assay delivers:

- Rapid setup — pre-verified, ready to use, and compatible with Universal Multiplex (UM) chemistry.
- Consistent performance — sensitive enough to detect rare molecules, precise enough for reproducible results every time.

This assay is part of Countable Labs’ Community Assays, a collection of ready-to-run assays shared with the scientific community to accelerate discovery. For the Influenza SARS-CoV-2 assay, please contact us at hello@countablelabs.com for information on the full primer sequences. All performance data was generated using the Countable platform.

Targets

The Influenza SARS-CoV-2 assay is designed to detect SARS-CoV-2 *NI*, Influenza A *M1*, Influenza B *NS2*, and human *RPP30* genes with the following specifications on the Countable platform.

Targets	Amplicon length	UM probe
SARS-CoV-2 <i>NI</i>	92 bp	UM-1
Influenza A <i>M1</i>	104 bp	UM-2
<i>RPP30</i>	65 bp	UM-3
Influenza B <i>NS2</i>	103 bp	UM-4

Control sample

The following synthetic dsDNA templates spiked into genomic DNA from human cell line GM12878 were used to establish the Influenza SARS-CoV-2 assay performance and is also recommended to use as a Training Sample during your Countable PCR setup. While not required for running the assay, using this control helps:

- Improve the specificity of your counts
- Serve as a quick check that your setup is performing optimally
- Provide a reference point for monitoring consistency across runs

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> Template_sequence SARS-CoV2N1
GAAGAAGGCTGATGAACTCAAGCCTTACCGCAGAGACAGAAGAAACAGCAAACCTGTGACTCTTCTTCTGCTGCAGATTGGAT
GATTTCTCCAAACAATTGCAACAATCCATGAGCAGTGCTGACTCAACTCAGGCCTAAACTCATGCAGACCACACAAGGCAGATGG
GCTATATAAACGTTTTTCGCTTTTCCGTTTACGATATATAGTCTACTCTTGTGCAGAATGAATTCTCGTAACTACATAGCA
> Template_sequence InfluenzaA M1
TTTCTATCATCCCGTCAGGCCCCCTCAAAGCCGAGATCGCGCAGAGACTGGAAAGTGTCTTTGCAGGAAAGAACACAGATCTTGA
GGCTCTCATGGAATGGCTAAAGACAAGACCAATCTTGTACCTCTGACTAAGGGAATTTAGGATTTGTGTTACAGCTCACCGTG
CCCAGTGAGCAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCTAAATGGGAATGGGGACCCGAACAACATGGATAG
> Template_sequence InfluenzaB NS2
ATGCATATGACAGAAATGGAGGGCTTGTGCTAACTTGTGCTACTGATGATCGGACAGTGGAGGATGAAAAGATGGCCATCG
GATCCTCAACTCACTCTTCGAGCGTTTGTGATGAAGGACATTCAAAGCCAATTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCC
CAATTTGGTCAAGAGCACCATTATCACCAGAAGAGGGAGACAATTAGACTGGCCACGGAAGAAGCTTTATCTCTTGAGTA
```

PERFORMANCE DATA

Assay signal distribution

Clear separation between the target signal and the background is critical for accurate calls. The Influenza SARS-CoV-2 assay consistently achieves an Intensity Distribution (ID) score above 90 — signifying that the data has a clean distinction between “signal” and “noise,” reducing false positives and improving confidence in quantification.

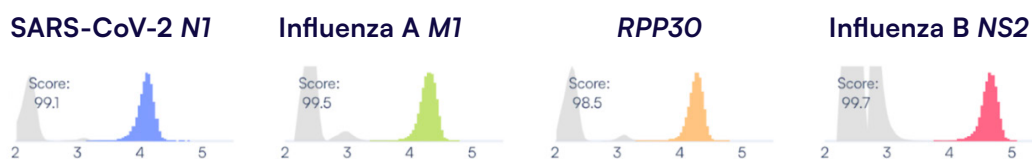


Figure 1. Representative fluorescence intensity histogram for the Countable Influenza SARS-CoV-2 assay, showing clear separation between target signal (SARS-CoV-2 N1: blue; Influenza A M1: green, RPP30: orange; Influenza B NS2: red) and background (grey). The Intensity Distribution (ID) score for each target appears in the upper-left corner, demonstrating strong signal-to-noise performance.

Instant multiplexing

The Influenza SARS-CoV-2 assay demonstrates consistent multiplex performance, yielding equivalent Counts per 50 µL whether targets are run individually (1-plex) or together (4-plex). This robust performance saves you time and ensures reliable results when running multiple targets.

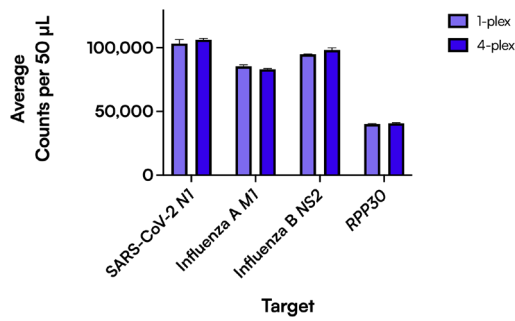


Figure 2. Consistent multiplex assay performance of the Countable Influenza SARS-CoV-2 assay. Bars represent individual Counts per 50 µL for each reaction, amplified using the synthetic training sample with either a single (1-plex; n=3) or four primer pairs (4-plex; n=3).

Linearity and sensitivity

A dilution series of the control sample shows excellent linearity across a broad dynamic range, demonstrating quantification abilities from high to very low target concentrations.

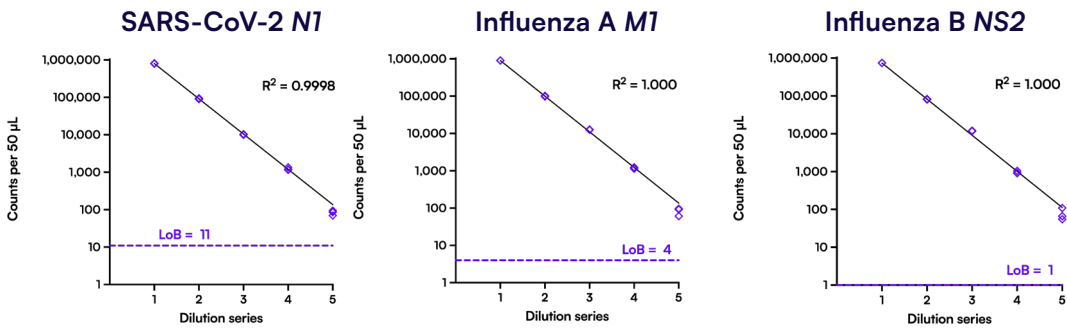


Figure 3. Linearity and limit of blank (LoB) for the Countable Influenza SARS-CoV-2 assay. A 5-fold dilution series of the control sample (n=4) shows excellent linearity ($R^2 = 0.9998, 1.0000, 1.0000$) across the dynamic range. The dashed line marks the LoB for each target, highlighting the assay's ability to detect even rare targets with accuracy.

Precision

Across the assay’s full dynamic range, the %CV remains low, delivering consistent and reproducible results. This precision enables confident detection of SARS-CoV-2 *N1*, Influenza A *M1*, Influenza B *NS2*, and *RPP30* across a wide range of inputs, ensuring sensitive and reliable biomarker measurement every time.

Target	Dilution	1	2	3	4	5
SARS-CoV-2 <i>N1</i>	Average counts	801,205	91,686	10,158	1,233	83
	% CV	1.53%	2.86%	2.11%	8.77%	14.66%
Influenza A <i>M1</i>	Average counts	904,438	99,864	12,609	1,186	83
	% CV	0.28%	2.68%	1.28%	4.48%	23.08%
Influenza B <i>NS2</i>	Average counts	743,234	81,852	11,843	957	77
	% CV	0.15%	1.73%	1.78%	7.55%	37.22%

Table 2. Influenza SARS-CoV-2 assay precision across the dynamic range.

Summary

The Influenza SARS-CoV-2 assay delivers sensitive, reproducible results across a wide dynamic range — giving you high-quality biomarker data from the very first run.

Influenza SARS-CoV-2 assay

Getting started

This protocol describes the setup and execution of the Countable Influenza SARS-CoV-2 assay for detecting SARS-CoV-2 *NI*, Influenza A *M1*, Influenza B *NS2*, and human *RPP30* genes on the Countable PCR platform using Universal Multiplex chemistry.

Targets	Amplicon length	UM probe
SARS-CoV-2 <i>NI</i>	92 bp	UM-1
Influenza A <i>M1</i>	104 bp	UM-2
<i>RPP30</i>	65 bp	UM-3
Influenza A <i>NS2</i>	103 bp	UM-4

Materials

Listed below are the materials needed for setting up the amplification mix of this specific assay. Refer to Countable PCR™ Reaction Preparation User Guide (IFU004) for the complete list of required materials to set up a Countable PCR reaction.

- ☐ **4X Countable PCR Mix** (Required)
Cat #: KT0004 (PR0004)
- ☐ **50X UM-1, UM-2, UM-3, UM-4 Probes** (Required)
Cat #: KT0005 (PR0006-PR0009)
- ☐ **Influenza SARS-CoV-2 primer mix** (Required)
Visit website for sequences
- ☐ **Control sample** (Optional *)
Visit website for sequences

* The use of a training sample in the Countable system enhances the specificity of counts, verifies assay performance, and can serve as a control, particularly for detecting rare molecules.

Countable PCR reaction set-up

The table below lists the setup of the amplification mix specific to this assay. Refer to Countable PCR™ Reaction Preparation User Guide (IFU004) for complete setup instructions.

Reagents	Cat #	Per 50 µL reaction	Final conc.
Nuclease-free water	—	To 50 µL	—
4X Countable PCR Mix	KT0004 (PR0004)	12.5 µL	1X
50X UM-1, -2, -3, -4 Probes	KT0005 (PR0006-9)	1 µL each	1X
50X primer mix*	—	1 µL	1X
Template	—	Variable	—

* Refer to IFU004, Appendix E for details

Thermal cycling conditions

Ensure ramp rate setting of 2 °C/sec. Set the sample volume to 125 µL and the heated lid to 105 °C.

Cycle	Step	Temp (°C)	Time (mm:ss)
1	Initial denaturation	95 °C	02:00
30	Denaturation	95 °C	00:20
	Annealing & extension	60 °C	01:00
1	Hold	8 °C	∞