

Notices

Limitations of use

For research use only. Not for use in diagnostic procedures. Unless otherwise agreed to in writing, IDT does not intend these products to be used in clinical applications and does not warrant their fitness or suitability for any clinical diagnostic use. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

Safety data sheets pertaining to this product are available upon request.

Safety notices



Reminder symbols call attention to minor details that may be easily overlooked and compromise the procedure resulting in decreased assay performance.



Caution symbols denote critical steps in the procedure where risk of protocol failure or damage to the product itself could occur if not carefully observed.



Stop symbols indicate where this procedure may be safely suspended and resumed at a later time without risk of compromised assay performance. Make note of these steps and plan your workflow accordingly.

Revision history

Version	Release Date	Description of changes
Rev 01	June 6, 2024	Original protocol version
Rev 02	October 2025	InfA (H5) positive sample control, "1 X 10 ⁴ " corrected to "1 X 10 ³ "

Avian Influenza Type A (H5) Primers and Probe Set

Targets Influenza A Clade 2.3.4.4b

Reagents

Avian Influenza Type A (H5) Primers and Probe Set

- Part Number 10020786

Kit Contents

Component	Quantity (μL)	Storage (°C)	Content
InfA (H5) Primer/probe mix (200 rxns)	250	-20	20X primer/probe stock solution targeting InfA H5 (FAM)
<i>InfA (H5) PCR control assay (200 rxns)</i>	250		20X Primer/Probe stock solution targeting InfA PCR control (SUN) InfA (H5) PCR synthetic control (5 × 10 ³ copies/μL)

Additional reagents (not included)

Component	Quantity	Manufacturer	Catalog #
PrimeTime 1-Step 4X Broad-Range Master Mix	1 mL	IDT	10011744
<i>Nuclease Free Water</i>	10 x 2 mL	IDT	11-04-02-01
<i>InfA (H5) positive sample control</i>	250 μL (1 × 10 ³ copies/μL)	IDT	10020808
<i>Mycoplasma BVD Milk Extraction Kit</i>		Udder Health Systems*	EXT-BVD1

*<https://www.udderhealth.com>

Milk Sample Lysis Protocol

1. For each sample, combine 49.5 μ L of the lysis buffer and 5.5 μ L of the enzyme mix in a clean 1.5 mL tube. Vortex and centrifuge for 5 minutes at 16300 RCF.
2. Dispense 50 μ L into a 200 clear strip tube for each sample.
3. Add 25 μ L of milk for each sample to the appropriate tube, capping the tubes as you go.
4. Vortex and spin down the tubes to collect the liquid.
5. Heat the tubes to 60 °C for 30 minutes.
6. Heat the tubes to 95 °C for 15 minutes.
7. Allow the tubes to cool to 60 °C or below.
8. Spin the tubes down for 5 minutes at 16300 RCF to pellet the solid material.
9. Use 5 μ L of the aqueous layer as sample in a 20 μ L reaction. For best results, prepare fresh samples for each reaction rather than storing the crude lysates.

Table 1. Positive control RT-qPCR setup

Component	Volume (μ L)
InfA (H5) Primer/probe mix	1
InfA (H5) PCR control	1
PrimeTime 1-Step 4X Broad-Range Master Mix	5
InfA (H5) positive sample control	1
Nuclease-free water	12
Total	20

Table 2. Crude lysate RT-qPCR setup

Component	Volume (μ L)
InfA (H5) Primer/probe mix	1
InfA (H5) PCR control	1
PrimeTime 1-Step 4X Broad-Range Master Mix	5
Crude lysis sample	5
Nuclease-free water	8
Total	20

Table 3. RT-qPCR cycling protocol

Step	Cycles	Temperature (°C)	Time
Reverse transcription	1	50	15 min
Enzyme activation	1	95	3 min
Denaturation	40	95	15 sec
Annealing/extension	40	60	1 min

Run RT-PCR

Place the plate in the real-time PCR instrument and start the cycling program.

Find safety data sheets (SDSs) and certificate of analysis (COAs) for IDT products at:

- <https://www.idtdna.com/pages/support/safety-data-sheets>
- <https://www.idtdna.com/pages/support/certificate-of-analysis-search>

For additional information or assistance, [Contact us](#).