Avian Influenza Type A (H5) Primers and Probe Set

Targets Influenza A Clade 2.3.4.4b

IDT consumables (kit 10020786 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)
InfA (H5) PCR control assay (200 rxns)	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
Nuclease Free Water	10 x 2 mL	IDT	11-04-02-01
InfA (H5) positive sample control	250 µL (1×10^4 copies/µL)	IDT	10020808
Mycoplasma BVD Milk Extraction Kit	—	Udder Health Systems*	EXT-BVD1

*https://www.udderhealth.com

Milk sample lysis

1. For each sample, combine 49.5 µL of the lysis buffer and 5.5 µL of the enzyme mix in a clean 1.5 mL tube. Vortex and centrifuge for 5 minutes at 16300 RCF.
2. Dispense 50 µL into a 200 clear strip tube for each sample.
3. Add 25 µL 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 µL of the aqueous layer as sample in a 20 µL 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 (µL)
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 (µL)
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.

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