Swine Influenza Virus RNA Test Kit

VetMAX™-Gold SIV Detection Kit

Catalog Number 4415200

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product information

Name, intended use, and principle of the procedure

The Swine Influenza Virus RNA Test Kit is a highly sensitive, qualitative, one-step, real-time reverse transcription PCR (real-time RT-PCR) assay to detect Swine Influenza Virus (SIV) RNA isolated from porcine nasal swab samples.

SIV is an enveloped, negative-sense RNA virus of the *Influenzavirus A* genus and is a member of the family *Orthomyxoviridae*. The nucleoprotein and matrix genes are used to identify SIV. SIV subtypes are defined by the surface glycoproteins: hemagglutinin and neuraminidase, with H1N1, H3N2, and H1N2 representing the predominant subtypes in swine. Swine influenza is an acute, infectious, and highly contagious febrile respiratory disease found in swine. The disease causes high morbidity and low mortality, and is characterized by sudden onset, coughing, dyspnea, fever, and prostration. SIV is a major contributor to the porcine respiratory disease complex (PRDC), resulting in significant economic losses in the swine industry. The VetMAX™-Gold SIV Detection Kit enables diagnosis of influenza in swine.

The assay is a single-well/tube, real-time RT-PCR in which RNA is reverse-transcribed into cDNA, and SIV and Xeno™ RNA targets are amplified and detected in real time using fluorescent TaqMan® probes (hydrolysis probe chemistry). The kit includes:

- Influenza Virus- Xeno™ RNA Control Mix: serves as a positive control for the real-time RT-PCR components and it is also used to set the cycle threshold (C_T) for evaluating test results.
- Xeno™ RNA Control: serves as an internal positive control for the RNA purification process and monitors for the presence of PCR inhibitors.
- Influenza Virus Primer Probe Mix for optimized multiplex real-time RT-PCR amplification of Xeno™ RNA Control and SIV RNA targets.

Limitations

- The kit is not intended for differentiating SIV subtypes.
- Handle samples as recommended in Table 1 to prevent degradation of any SIV RNA that is present.
- RNA extraction methods should yield RNA free of RT-PCR inhibitors, which can prevent amplification of target RNA.
- Follow "Good laboratory practices for PCR and RT-PCR" on page 5 to prevent false positive amplifications due to contamination of test samples with PCR products.

Kit contents and storage conditions

Reagents for 100 25-µL real-time RT-PCR tests are supplied.

Component	Volume	Storage
2X Multiplex RT-PCR Buffer	1375 µL	–30°C to –10°C
Multiplex RT-PCR Enzyme Mix	280 µL	–30°C to –10°C
Influenza Virus Primer Probe Mix	110 µL	–30°C to –10°C
Xeno™ RNA Control (10,000 copies/µL)	250 µL	–30°C to –10°C
Influenza Virus-Xeno™ RNA Control Mix (1000 copies/µL)	80 µL	–30°C to –10°C
Nuclease-free Water	1.75 mL	–30°C to +25°C

Required materials not supplied

Item	Source
Plates or tubes appropriate for the Applied Biosystems® 7500 Fast Real-Time PCR System (96-well)	• MicroAmp® Optical 8-Cap Strip (Cat. no. 4323032), or equivalent • MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1-mL (Cat. nos. 4366932, 4346906), or equivalent. • MicroAmp® Optical Adhesive Film (Cat. nos. 4311971, 4360954), or equivalent • MicroAmp® Fast 8-Tube Strip, 0.1-mL (Cat. no. 4358293), or equivalent • Precision Plate Holder for 0.1-mL tube strips (Cat. no. 4403809), or equivalent
Nuclease-free pipettes and filtered pipette tips	Major laboratory supplier (MLS)
Nuclease-free reagent tubes for preparing master mixes	MLS
Real-time PCR thermal cycler	Applied Biosystems® 7500 Fast Real-Time PCR System (96-well), running SDS software v1.4
1X Phosphate Buffered Saline (PBS), pH 7.4	MLS
Viral Transport Media	MLS
2 ice buckets: • One for the PCR setup area where the master mix is prepared • One for the area where RNA may be present	MLS

For Veterinary Use Only.

Isolate RNA from samples

Table 1 Sample handling recommendations

Step or process	Recommendation
Transport/storage of samples	Transport nasal swabs samples at 4°C to 25°C.
Preparation of swab samples	1. Place one nasal swab sample into a 1.5-mL tube or deep-well 96-well plate, then add 0.75 mL of Viral Transport Media. 2. Vortex vigorously for 3 minutes, then pulse-spin to remove debris from the tube cap. 3. Remove 50 µL of supernatant for RNA isolation.
Preparation of mock-purified samples (for use in extraction control PCRs)	Prepare duplicate mock-purified samples, using 1X PBS as the starting material. Process with the same RNA isolation method that is used for test samples.
Proposed RNA isolation method	MagMAX™-96 Viral RNA Isolation Kit (Cat. nos. AM1836, AMB1836-5) or an equivalent RNA isolation method.
Required modifications to the RNA isolation method	• Add 2 µL of undiluted Xeno™ RNA Control per isolation to the lysis solution used for RNA isolation. • Add carrier RNA to the lysis solution according to the manufacturer recommendations. Carrier RNA is provided in the MagMAX™-96 Viral RNA Isolation Kit (Cat. nos. AM1836, AMB1836-5)

Perform real-time RT-PCR

1 Determine the quantity of reactions and thaw the reagents

a. On each plate, include the following control reactions (for step 4 of this procedure):
   - Positive control (prepare duplicate reactions); use 8 µL of the Influenza Virus-Xeno™ RNA Control Mix (1000 copies/µL).
   - No-template control (NTC) (prepare duplicate reactions); use Nuclease-free Water in place of sample RNA.

b. Plan the plate layout so that the wells containing NTCs are located as far as possible from positive controls and test samples to prevent accidental cross-contamination.

c. Thaw RT-PCR master mix reagents in one ice bucket and controls and samples in a separate ice bucket, gently vortex each tube to mix the contents thoroughly, then briefly centrifuge to collect the solution at the bottom of the tube. Keep the reagents on ice.

2 Prepare the RT-PCR master mix on ice

Combine the following components for the number of reactions required plus 10% overage.

Component	Volume per reaction
2X Multiplex RT-PCR Buffer	12.5 µL
Multiplex RT-PCR Enzyme Mix	2.5 µL
Influenza Virus Primer Probe Mix	1.0 µL
Nuclease-free Water	1.0 µL
Total volume of RT-PCR master mix	**17.0 µL**

3 Set up the RT-PCR reactions

a. Dispense 17 µL of RT-PCR master mix to the appropriate wells of a PCR plate or PCR tubes on ice.

b. Add the appropriate component for the reaction type, according to the following table:

Reaction type	Component	Volume per reaction
Test sample	Sample RNA	8.0 µL
NTC	Nuclease-free Water	8.0 µL
Positive control	Influenza Virus-Xeno™-RNA Control Mix (1000 copies/µL)	8.0 µL
Extraction control	Mock-purified PBS sample	8.0 µL

c. Seal each reaction vessel, mix, then centrifuge briefly to bring the contents to the bottom.

4 **Set up and run the real-time PCR instrument**

a. Following the manufacturer's instructions, set up the run using the following parameters:
- Run mode: Standard 7500
- Reaction volume: 25 µL
- ROX™ passive reference dye: Included in the RT-PCR Buffer
- TaqMan® probe reporter dyes and quenchers:

Target	Reporter	Quencher
SIV RNA	FAM™ dye[1]	Eclipse® Q
Xeno™ RNA Control	VIC® dye[2]	Eclipse® Q

[1] Absorbance maximum of 495 nm; emission maximum of 520 nm.
[2] Absorbance maximum of 540 nm; emission maximum of 552 nm.

b. Run the thermal cycler program and collect real-time amplification data during stage 3. Use the following thermal cycler settings:

Stage		Reps.	Temp.	Time
Reverse transcription	1	1	48 °C	10 minutes
RT inactivation/initial denaturation	2	1	95 °C	10 minutes
Amplification	3	40	95 °C 60 °C	15 seconds 45 seconds

Data analysis

Refer to your real-time PCR instrument user guide for instructions on how to analyze your data, using the following method.

Table 2 Data analysis

Method	Details
Use the **Control-Based Threshold** setting for data analysis.	1. Select **Manual C_T**. 2. Export ΔRn values for the positive control samples (Influenza Virus-Xeno™ RNA Control, 1000 copies/µL). 3. Average the FAM™ and VIC® values (separately) for the ΔRn at cycle 40 for all replicates of the positive control reaction. 4. Set the threshold for the SIV RNA reactions at 5% of the average maximum fluorescence value of the SIV RNA amplification signal in the positive control reactions. Example: If the average maximum fluorescence value for the SIV RNA target in the positive control reactions is 3.0, set the SIV RNA threshold at 0.15. 5. Repeat step 4 for the Xeno™ RNA Control target using a 5% threshold. Example: If the average maximum fluorescence value for the Xeno™ RNA target in the positive control reactions is 2.0, set the Xeno™ RNA threshold at 0.1.
Check the raw fluorescence data.	Verify that increased fluorescence seen in the normalized data is also evident without mathematical data processing.

Interpretation of test results

Table 3 Criteria for a valid real-time RT-PCR run

Reaction type	C_T value for SIV RNA	C_T value for Xeno™ RNA Control
Positive control	25–29	25–29
NTC	Not detected[1]	Not detected[1]
Extraction control	Not detected[1]	27.5–34

[1] If the C_T value is <40, see "Troubleshooting" on page 4.

Table 4 Interpretation of sample test results

C_T value for SIV RNA	C_T value for Xeno™ RNA	Interpretation
< 38	27.5–34[1]	SIV-positive sample
Not detected	27.5–34	SIV-negative sample
38 to 40	27.5–34[2]	Suspect result

[1] See "Troubleshooting" on page 4.
[2] See Table 5.

Table 5 Assessment of suspect results

Result	Action	
Suspect result 1: The sample SIV C_T value is <40 and the C_T value for one or more NTCs or extraction controls is <40.	Repeat the extraction or real-time RT-PCR. See "NTC or extraction control reaction" on page 5.	
Suspect result 2: The sample SIV C_T value is 38 to 40.	Analyze suspect RNA samples for the presence/absence of RT-PCR inhibitors by calculating the Xeno™ RNA C_T shift: **Xeno™ RNA C_T Shift = SS – XEC**, where: **SS =** C_T of Xeno™ RNA in the suspect sample **XEC =** Average C_T of Xeno™ RNA in the extraction controls	
	Workflow A **Xeno™ RNA C_T shift ≥1.5**	**Workflow B** **Xeno™ RNA C_T shift <1.5**
	1. Repeat the real-time RT-PCR with 2 µL of the suspect RNA sample. (RT-PCR inhibitors may be present in the RNA.) If the SIV C_T value is: • <38 – The sample is SIV positive. No further testing is required. • ≥38 – Continue with steps 2 through 5 of this procedure. 2. Dilute the original diagnostic sample 1:4. 3. Repeat the RNA purification on triplicate aliquots of the diluted sample. 4. Repeat the real-time RT-PCR with 8 µL of purified RNA from step 3. 5. Determine the number of samples with a SIV C_T value <40: • 0 of 3: SIV negative • 1 of 3: Presumptive positive; confirm with secondary test method • ≥2 of 3: SIV positive	1. Repeat the RNA purification on triplicate aliquots of the original diagnostic sample. 2. Repeat the real-time RT-PCR with 8 µL of purified RNA from step 1. 3. Determine the number of samples with a SIV C_T value <40: • 0 of 3: SIV negative • 1 of 3: Presumptive positive; confirm with secondary test method • ≥2 of 3: SIV positive

Troubleshooting

Observation	Possible cause	Recommended action
Positive control reaction Influenza Virus-Xeno™Control RNA—no signal Xeno™ RNA Control—no signal	The Influenza Virus-Xeno™ RNA Control Mix was improperly handled, resulting in RNA degradation.	Use appropriate precautions against RNase contamination when handling the control RNAs. For example, wear clean gloves and use nuclease-free barrier pipette tips.
	The Multiplex RT-PCR Enzyme Mix was stored or handled improperly, and it lost activity.	Repeat the RT-PCR with fresh reagents.
	The thermal cycler was not properly set up.	Check the thermal cycler settings. See "Set up and run the real-time PCR instrument" on page 3.
	The RT-PCR master mix was prepared incorrectly.	Repeat the test with correctly prepared RT-PCR master mix.

Observation	Possible cause	Recommended action
NTC or extraction control reaction C_T value is < 40	There was contamination during the RNA extraction or PCR.	• Repeat the RNA isolation or real-time RT-PCR with fresh reagents and freshly decontaminated pipettes. • Set up the real-time RT-PCR in an area separate from areas used for RNA isolation and PCR product analysis.
Test samples Xeno™ RNA Control—no or low signal SIV RNA—high signal	The Xeno™ RNA Control primers and probe are at limiting concentrations in the RT-PCR. High levels of SIV RNA in a sample can reduce amplification of Xeno™ RNA Control.	No or low signal from Xeno™ RNA Control is expected in a reaction that has a strong signal for SIV RNA.
Test samples Xeno™ RNA Control—no signal SIV RNA—no signal or suspect-range signal	Poor RNA recovery.	Check the C_T values of Xeno™ RNA Control in the mock-purified samples. $C_T \geq 38$: indicates that Xeno™ RNA Control was omitted or that RNA recovery was poor. Repeat the RNA purification of the original diagnostic sample.
	The RNA samples contain inhibitors of RT-PCR.	See Table 5.

Explanation of symbols

The symbols present on the product label are explained in the following table.

	MANUFACTURER		USE BY
REF	CATALOG NUMBER		CONSULT INSTRUCTIONS FOR USE
LOT	BATCH CODE		CAUTION, CONSULT ACCOMPANYING DOCUMENTS
SN	SERIAL NUMBER		UPPER AND LOWER LIMITS OF TEMPERATURE

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA*Zap*™ Solutions (Cat. no. AM9890).

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