



G4 EA H1N1 Swine Flu Virus RT-qPCR Detection Kit (G4EASF)

Catalog #RU7068

100 reactions

Product Description

Pigs have been shown to be key hosts for the generation of pandemic influenza viruses including the one that caused the 2009 H1N1 pandemic. Since 2016, a recently identified H1N1 swine flu virus genotype, the genotype 4 reassortant Eurasian avian-like (G4 EA) H1N1 virus, has rapidly spread and become the predominant genotype in the surveilled pig populations in China. The G4 EA H1N1 swine flu virus also demonstrated human infectivity and has the potential to become a pandemic virus.

ScienCell's G4 EA H1N1 Swine Flu Virus RT-qPCR Detection Kit (G4EASF) is designed to detect the presence of influenza A viruses and the newly emerged genotype G4 EA H1N1 Swine Flu virus in extracted RNA samples. Three primer/probe sets are included in the kit. Two of them (Cat #7068-InfA and 7068-G4EA) target a universal region shared by all influenza A viruses and a region specific to G4 EA H1N1 virus, respectively. The human ACTB gene primer/probe set (Cat #7068-ACTB) targets the human β -actin (ACTB) housekeeping gene, which serves as an internal control to assess specimen quality. All primer/probe sets are verified to possess high specificity and efficiency near 100% under recommended PCR conditions. In addition, a non-infectious positive control (Cat #7068-Pos) and nuclease-free water are included in the kit. The positive control (Cat #7068-Pos) consists of non-infectious viral RNA fragments and serves to ensure reagents and instruments are working properly. Please refer to Tables 4 and 5 for results interpretation.

Kit Components

Cat #	Component	Quantity	Storage
MB802a	One-Step TaqProbe RT-qPCR master mix, 4x	1.5 mL	-20°C
7068-InfA	Universal influenza A primer/probe set, in solution	200 μ L	-20°C
7068-G4EA	G4 EA primer/probe set, in solution	200 μ L	-20°C
7068-ACTB	Human ACTB gene primer/probe set, in solution	200 μ L	-20°C
7068-Pos	Positive control (non-infectious RNA: 500 – 1000 copies/ μ L)	200 μ L	-80°C
GQ100-4	Nuclease-free H ₂ O	4 mL	4°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
RNA Isolation Kit	ScienCell Viral RNA Isolation Kit (ScienCell, Cat #MB891)
qPCR plate or tube	

Quality Control

The primer/probe sets and the positive control are validated by RT-qPCR using serially diluted templates. The PCR products are analyzed by gel electrophoresis.

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Shipping and Storage

The product is shipped on dry ice. Upon receipt, store the One-Step TaqProbe RT-qPCR master mix (Cat #MB802a) and primer/probe sets (Cat #7068-InfA, 7068-G4EA, and 7068-ACTB) at -20°C in a manual defrost freezer, the positive control (Cat #7068-Pos) at -80°C, and nuclease-free H₂O (Cat #GQ100-4) at 4°C. Aliquot as needed. Avoid repeated freeze-and-thaw cycles.

Procedures

Important: Only use nuclease-free reagents in PCR applications.

Note: This master mix does not contain a ROX passive reference dye. If the qPCR instrument being used has a "ROX passive reference dye" option, please deselect this option.

1. Prior to use, allow the primer/probe sets (Cat #7068-InfA, 7068-G4EA, and 7068-ACTB) to thaw to room temperature in the dark. Shake gently to mix well.
2. Centrifuge the vials at 1,500x g for 1 minute.
3. Aliquot each primer/probe set as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles. Keep on ice when thawed.
4. For the test samples, two control samples should be run concurrently: the non-infectious positive control (Cat #7068-Pos) and H₂O (Cat #GQ100-4) as the No Template Control (NTC). Prepare three RT-qPCR reactions for each control sample, one with #7068-InfA primer/probe set, one with #7068-G4EA primer/probe set, and one with #7068-ACTB primer/probe set. Prepare 20 µl RT-qPCR reactions for one well as shown in Table 1.

Table 1.

Control sample (Cat #7068-Pos or GQ100-4)	5 µl
Primer/probe set (Cat #7068-InfA, 7068-G4EA, or 7068-ACTB)	2 µl
One-step RT-qPCR Master mix, 4x (Cat #MB802a)	5 µl
Nuclease-free H ₂ O (Cat #GQ100-4)	8 µl
Total volume	20 µl

5. For each extracted RNA test sample, prepare three RT-qPCR reactions, one with #7068-InfA primer/probe set, one with #7068-G4EA primer/probe set, and one with #7068-ACTB primer/probe set. Prepare 20 µl RT-qPCR reactions for one well as shown in Table 2.

Table 2.

RNA test sample (concentration varies)	5 µl
Primer/probe set (Cat #7068-InfA, 7068-G4EA, or 7068-ACTB)	2 µl
One-step RT-qPCR Master mix, 4x (Cat #MB802a)	5 µl
Nuclease-free H ₂ O (Cat #GQ100-4)	8 µl
Total volume	20 µl

6. Seal the RT-qPCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds.
7. Setup RT-qPCR reactions as shown in Table 3.

Table 3. Instrument settings for RT-qPCR reactions.

Fluorescence data (FAM) should be collected during the data acquisition step.

Step	Temperature	Time	Number of cycles
UNG incubation	25°C	2 min	1
Reverse transcription	50°C	20 min	1
Taq DNA polymerase activation	95°C	10 min	1
Denaturation	95°C	20 sec	40
Annealing	65°C	20 sec	
Extension	72°C	20 sec	
Data acquisition	Plate read, detector (FAM)		

Results Interpretation

Table 4. Interpretation of G4EASF kit control sample test results.

Sample	7068-InfA	7068-G4EA	7068-ACTB	Results Interpretation
7068-Pos	+	+	+	Expected
	-	-	-	Reverse transcription and/or PCR failed
GQ100-4	-	-	-	Expected
	If anyone of three targets is positive			Reagent(s) contaminated

Note: Cq values less than 35 are considered positive. Cq values equal to or greater than 35 are considered negative.

Table 5. Interpretation of G4EASF kit target sample test results when control results are as expected.

7068-InfA	7068-G4EA	7068-ACTB	Results Interpretation
±	+	±	G4 EA H1N1 virus detected
+	-	±	Influenza A virus but not G4 EA detected
-	-	+	No influenza A virus detected
-	-	-	Invalid result

Note: Cq values less than 35 are considered positive. Cq values equal to or greater than 35 are considered negative.