

VetDetect H5

Cat. No. V02-01-1121

One-step real-time reverse transcription PCR for
detection of avian influenza virus subtype H5

Includes main components for 50 reactions

LightCycler Version



Rev. 0
December 2005
Hong Kong DNA Chips Ltd

1. KIT COMPONENTS

PLEASE READ THROUGH THE ENTIRE PROTOCOL BEFORE STARTING.

The kit contains reagents for a total of 20 reactions

Amplification reagents

- 20 Tubes Reagent Spheres (store at 4°C)
- 2 x 450µl H5 Sphere Diluent (store at -20°C)
- 2 x 25µl Positive Control (store at -20°C)

Note: The real-time PCR reagent mix **DOES NOT CONTAIN ROX** as passive reference.

Storage conditions

Store Reagent Spheres at 4°C with silica gel desiccant. Store Sphere Diluent, Positive Control, and reconstituted sphere at -20°C. Thaw frozen reagents just before use. Mix reagents thoroughly (do not vortex reconstituted sphere containing enzyme).

2. PROCEDURE

Mastermix Preparation

1. Determine required number of reactions (n).
2. Number of Reagent Spheres required = 0.4 x n
3. Add 40 µl Sphere Diluent for each Reagent Sphere.
4. Allow Reagent Sphere to reconstitute on ice.
5. Gently tap tubes to mix reagents. Do not vortex enzyme containing reagents

Set-up

1. In addition to the RNA obtained from the test samples, each experiment requires a positive and negative (water) control.
2. Set up real-time PCR components according to the table below:

Components	Volume per reaction
Mastermix	16 µl
Sample RNA	4 µl
Total Volume	20 µl

Note:

- Use real time PCR tubes and consumable recommended for your real-time PCR equipment.
- Keep RNA samples on ice throughout experiment.
- 4 µl of provided Positive Control can be used to monitor the success of amplification.
- It is advisable to run samples in duplicate to ensure reliability of results.
- Spiking Control: 1 µl of Positive Control can be spiked into test sample to check whether the test sample contains PCR inhibitory substances.

Cycling conditions:

		Data Collection Points (FAM Filter)
1 Cycle	42°C 30 Minutes	-
	95°C 10 Minutes	-
40 Cycles	95°C 15 Seconds	-
	58°C 60 Seconds	□

(Take readings at point □, set ramp rate to 5°C/second)

3. DATA ANALYSIS AND INTERPRETATION

Detailed explanations of the basic and advanced operating procedures should be provided with your LightCycler equipment.

Spiking control

Negative real-time PCR result may be due to a few scenarios: 1. absence of detected sequence in the sample; 2. presence of detected sequence below limit of detection; 3. presence of PCR inhibitory substances. The purpose of spiking control is to verify whether the test sample contains substances, which may affect PCR reactions. If Ct equals 40 (or similar to Ct given by negative control) when the test sample is spiked with the positive control, the test sample is highly likely to contain PCR inhibitory substances, and the result should NOT be taken as negative. Repeated extraction and real-time PCR of the sample will be required.

If you require more detailed analysis information, please contact Hong Kong DNA Chips for technical assistance.

4. TECHNICAL ASSISTANCE

Our technical staff will provide technical assistance you may need in using this kit. Simply call +(852) 2111 2123 during office hours:

Monday – Friday: 9:00am to 5:30pm
Saturday: 9:00am to 1:00pm

A recorded message (in English, Cantonese or Putonghua) may be left outside office hours.

Alternatively, you may contact our technical staff by fax or email.

Fax: +(852) 2111 9762
Email: technical@dnachip.com.hk

5. Warranties and liabilities

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