

# VetScreen AIV H5

Cat. No. V03-01-1111

One-step reverse transcription PCR for gel-based detection of Avian Influenza Virus (Subtype H5) in veterinary and environmental samples

Includes main components for 50 reactions



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Hong Kong DNA Chips Ltd

## 1. KIT COMPONENTS

*PLEASE READ THROUGH THE ENTIRE PROTOCOL BEFORE STARTING.*

The kit contains reagents for a total of 50 reactions:

### DNA amplification reagents

- 25 Tubes Reagent Spheres (store at 4°C)
- 2 x 550 µl H5 Sphere Diluent (store at -20°C)
- 2 x 25 µl Positive Control (store at -20°C)

### 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 Sphere(s) required =  $0.5 \times n$ .
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 PCR components according to the table below:

Components	Volume per reaction
Mastermix	20 µl
Sample RNA	5 µl
Total Volume	25 µl

### Note:

- 5 µl of the provided Positive Control can be used to monitor the success of amplification.
- Keep RNA samples on ice throughout experiment.
- It is advisable to run samples in duplicate to ensure reliability of results.
- Spiking Control: 1 µl Positive Control can be spiked into test sample to check whether the test sample contains PCR inhibitory substances.

### Cycling conditions:

1 Cycle	42°C	30 Minutes
	95°C	10 Minutes
40 Cycles	95°C	30 Seconds
	53°C	30 Seconds
	72°C	30 Seconds

## 3. DATA ANALYSIS AND INTERPRETATION

Expected PCR product size: 228 base pairs

### Spiking control

Negative 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 no band is visible on agarose gel 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 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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