VDS
AIV H7

User Manual
Cat. No. V01-01-1112

NASBA for the detection of avian influenza virus H7 subtype
in veterinary and environmental samples

To be used in conjunction with the
NucliSens Electrochemiluminescent Reader

Includes main components for 50 reactions

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HAI KANG LIFE CORPORATION LIMITED

PLEASE READ THROUGH THE ENTIRE PROTOCOL BEFORE STARTING.

1. KIT COMPONENTS

Storage Conditions
Components should be stored at -20°C with the exception of the small inner box, which should be stored at 4°C. Expiry dates shown on the box indicate the date beyond which reagents should no longer be used.

Nucleic Acid Amplification

- 5 x 6.5 mg Enzyme spheres (red cap; contained in a foil pack with silica gel desiccant)
- 1 x 0.5 ml Enzyme sphere diluent (red cap)
- 5 x 10 mg Reagent spheres (blue cap; contained in a foil pack with silica gel desiccant)
- 1 x 0.6 ml Reagent sphere diluent (blue cap)
- 1 x 0.5 ml KCl stock solution (yellow cap)
- 1 x 60 µl H7 primer mix
- 1 x 25 µl H7 positive control
- 2 x 1.5 ml NASBA water (white cap)

Nucleic Acid Detection (Small Inner Box Contents; store at 4°C)

- 1 x 0.9 ml Generic ECL detection probe (red cap)
- 1 x 0.65 ml H7 capture probe
- 2 x 1.7 ml Instrument Reference Solution (clear cap)

2. PREPARATION OF REAGENTS

A. Nucleic Acid Amplification

- Re-use of reagents: The reconstituted reagent sphere/enzyme sphere can be re-used once within two weeks of opening provided they have been stored at -70°C. Re-use of all other amplification reagents is possible if the remainder of the opened reagents has been stored at -20°C.
- Bring all reagents to room temperature before use.

Preparation of Amplification Solution

- Add 80 µl **reagent sphere diluent** to a lyophilised **reagent sphere** and immediately vortex well.
- Add 14 µl **NASBA water** and 16 µl **KCl stock solution** and vortex.
- Add 10 µl **H7 primer mix** and vortex.
- **DO NOT** centrifuge.
- Use within 30 min.

Preparation of the Enzyme Solution

- Add 55 µl **enzyme diluent** to a lyophilised **enzyme sphere**. Leave this enzyme solution to stand for at least 20 min at room temperature.
- Mix gently by flicking the microfuge tube with your finger.
- **DO NOT** vortex any solution containing enzymes.
- Centrifuge briefly before use.
- Use within one hour.

B. Nucleic Acid Detection

- Re-use of detection reagents is possible if the remainder of opened reagents has been stored at 2 - 8°C.
- Bring all reagents to room temperature before use.
- Spin down all microfuge tubes to bring liquid to the bottom of the tube.

C. Preparation of Hybridisation Solution

- Vortex **Generic ECL detection probe** and **H7 capture probe** until an opaque solution has formed.
- For *N* detection reactions, mix (*N*+2) x 10 µl H7 capture probe with (*N*+2) x 10 µl Generic ECL detection probe.
- Vortex hybridisation solution briefly before use.
- Use within one hour.

3. PROCEDURES

Remarks:

- Wear gloves throughout the procedure to protect your RNA samples from nucleases.
- All procedures should be carried out at room temperature unless otherwise stated.
- DO NOT vortex any solutions containing enzymes.

Nucleic Acid Amplification

1. For each amplification reaction, pipette 5 µl of the nucleic acid extract into a fresh microfuge tube.
2. Add 10 µl of **amplification solution** (see section 2).
3. Incubate microfuge tubes for 5 min at 65°C in a heat block.
4. Cool microfuge tubes for 5 min at 41°C in a heat block.
5. Add 5 µl of **enzyme solution** (see section 2) and mix well by flicking the microfuge tube with your finger.
6. Immediately return microfuge tubes to 41°C for 5 min.
7. Briefly centrifuge microfuge tubes and incubate for 90 min at 41°C in a water bath.
8. Perform the detection or store the RNA amplicons at -20°C for up to 1 month.

Nucleic Acid Detection

1. Take (*N*+2) 5 ml polypropylene tubes. Label all tubes. Keep Tube 1 for the **Instrument Reference Solution** (IRS) provided in the kit and Tube 2 for the blank control.
2. Vortex the **hybridisation solution** until opaque (see section 2) and add 20 µl solution to each of the tubes **except Tube 1**.
3. For blank control, add 5 µl **NASBA water** to tube 2.
4. For the sample reactions, add 5 µl RNA amplicons to tube 3, 4, etc.
5. Cover all tubes with parafilm and gently vortex all tubes until an opaque solution has formed.
6. Incubate all tubes for 30 min at 41°C. Gently vortex the tubes to mix every 10 min.
7. Add 0.3 ml **assay buffer** (provided with NucliSens Reader) to each tube **except Tube 1**.
8. Vortex the **IRS** until opaque and add 0.25 ml IRS to Tube 1.
9. Place the tubes on the appropriate positions of the instrument carousel.
10. Create a new run worksheet and input sample number (see section 4).

4. DATA COLLECTION, ANALYSIS AND INTERPRETATION

Detailed explanations of the basic and advanced operating procedures and equipment maintenance are provided in the NucliSens Reader Operator Manual.

Create a New Run

1. Switch on the NucliSens Reader computer.
2. On the *start-up* screen, click “Prime” to perform instrument calibration.
3. When instrument is ready click “start.” The calibration will take approximately 3 min.

4. Under user name on the *log-in* screen, select "service engineer" and click "log in." There is no need to enter a password.
5. Select "Routine" at the top left of the screen and select "New Run."
6. On the *flush the worklist* screen click "yes."
7. Enter a filename (up to 8 characters) and click "ok."
8. A worklist sheet will open. Under selected assay select "Free tube."
9. Enter sample ID and click "Add to list."
   Note: do not change the sample volume from the preset 100 µl.
10. Repeat sample ID input for all samples.
11. When finished, click "Close."
12. The sample worklist details will show. Check the worklist and click "ok."
13. Ensure your samples are placed on the carousel according to the sample ID order input to the computer.
14. Select "Routine" at the top left of the screen and select "Run Worklist."
15. A *check fluids* screen will appear. Click "Proceed."
16. The detection will start (each tube will take about 1.5 min).
17. After the detection stops, select "Routine" at the top left of screen and select "Assay result" to obtain results.

Results and Interpretation

The NucliSens Reader automatically measures the ECL signal of the hybridised samples and the user software calculates the corresponding result. For example, the results of detection will be displayed similar to the following (Note: The experimental data shown in the following table is for example only – your results will be different)

Table 1. Typical NucliSens results summary

Sample ID	Qualification	Tube	Det.	Qualitative Analysis	Signal (Detectable counts)
IRS	Valid	1	IRS		35,500
Assay negative	Valid	2	Free tube	Negative	138
1	Valid	3	Free tube	Positive	6,961
2	Valid	4	Free tube	Positive	10,494
3	Valid	5	Free tube	Positive	10,000,001
Negative control	Valid	6	Free tube	Negative	70

5. DETERMINING THE CUT-OFF LIMIT

The calculation of the most appropriate cut-off limit to adequately discriminate between genuine positive and negative samples is a relatively straightforward procedure. An example is provided here as a reference:

1. Test 5 known negative samples and 5 known "weak positive" samples (i.e. dilutions of known positives). NASBA reaction generally produces strong positive ECL signals with very few intermediate ECL signals.
2. The cut-off can be defined as a fraction of the Instrument Reference Solution (IRS), which is run at the beginning of every experiment. Typical values for IRS are 15,000 to 60,000 units.
3. Define a cut-off limit that can correctly differentiate the negative and weak positive samples tested above. The cut-off limit will be greater than the highest ECL signal obtained for a known negative sample and lower than the lowest ECL signal obtained for a known weak positive. Generally, a factor between 0.01 to 0.15 x IRS is appropriate (i.e. 1% to 15% of the IRS).
4. Further known negative and weak positive samples can then be tested to further qualify the appropriateness of the selected cut-off value.
5. The chosen cut-off value will be valid for the current set of experiments only and must be defined again when the assay is run in the future or if other parameters are altered (e.g. buffer composition, extraction method, length of incubation, etc).

Table 2. Summary of cut-off limit determination data

Negative sample	ECL signal
1	137
2	303
3	275
4	375
5	632
Negative control	289
Assay negative	127
IRS	36,345
Cut-off limit	
0.01 x IRS	363
0.015 x IRS	545
0.02 x IRS	727
0.025 x IRS	909
0.15 x IRS	5452

As the highest known negative sample has an ECL signal of 632, the most appropriate cut-off limit to select on this occasion would be 0.025 x IRS.

6. TROUBLESHOOTING GUIDE

Symptoms	Possible Causes	Problem solving
Low ECL signal	RNase contamination	The reagents provided are all RNase-free. All other solutions and apparatus used (pipettes, tips and microfuge tubes, etc) should be treated to ensure they are also RNase-free.
	Insufficient template / Degradation of nucleic acid extracts	RNA preparations should be stored properly to prevent degradation. Nucleic acid samples can be stored: ▪ long-term at -70°C ▪ up to 7 days at 2°C to 8°C ▪ up to 48 hours at 25°C
	Poor amplification efficiency	Add enzyme solution to the microfuge tubes by taking the microfuge tubes out of the heat block one at a time and returning them immediately after addition of enzyme. Maintain the amplification temperature at 41°C ±1°C
	Enzymes denatured	**DO NOT** vortex the enzyme solution. Enzymes are quite sensitive to physical influences and lose their activity when vortex mixed. **DO NOT** incubate the enzymes above 42°C. This will also denature enzyme activity.
	Poor hybridisation between target RNA and probe	Vortex the capture probe and generic probe tube until an opaque suspension is formed before preparing the hybridisation solution. Vortex the hybridisation solution and sample mixture regularly.
IRS reading below 10,000	Equipment not calibrated	Contact your bioMérieux representative.

7. 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@haikanglife.com

8. WARRANTIES AND LIABILITIES

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