Instruction for Use
K-Influenza A/B Antigen Test

1. Purpose of use

The K-Influenza A/B Antigen Test is intended for the detection of Influenza A and Influenza B nucleocapsid protein antigen in nasopharyngeal swab specimens directly collected from suspected flu infection within 7 days after symptom onset. This test kit aims for *in vitro* diagnostic only and is intended for professional use.

2. Test principle

The K-Influenza A/B Antigen Test is a colored chromatographic immunoassay. After extraction, the sample is dropped on sample pad, and the buffer flows through the conjugate pad containing anti-nucleocapsid conjugated gold nanoparticle. If the sample contains Influenza A/B viral nucleocapsid antigen, the antigen binds to the conjugate to form an anti-nucleocapsid gold conjugate – nucleocapsid complex. The complex flows to the test line coated with anti-nucleocapsid antibody, leading to the formation of an anti-nucleocapsid gold conjugate – nucleocapsid – anti-nucleocapsid complex, which will produce a visible color on the test line.

Positive results indicate the presence of Influenza A or Influenza B antigens in the tested specimen. To ensure the accuracy of the result, real time RT-PCR assay should be performed further as a confirmatory test.

Negative results indicate no Influenza A or Influenza B antigens in the tested specimen, but the possibility of influenza infection is not excluded. Duration of infection, patient history, signs and symptoms need to be considered for diagnosis.

3. Provided materials

The K-Influenza A/B Antigen Test for one box contains the following items:

1. Individually foil-pouched test cassettes, with a desiccant (25 packs)
2. Sample extraction buffer in a sample extraction tube (25 tubes)
3. Sterile nasopharyngeal swab (25 pieces)
4. Paper rack (1 piece)
5. Instruction for Use

4. Storage

Store the K-Influenza A/B Antigen Test at -30°C. Avoid conditions of high heat and humidity.

5. Specimen collection

1) Tilt the patient's head 70° backward. Hold nasopharyngeal swab perpendicular to the patient's face.
2) Gently insert the nasopharyngeal swab through nares parallel to palate until resistance is met or the posterior nasopharynx is reached (distance equivalent to half the distance from the ear to the nostril).
3) Gently rub and roll the nasopharyngeal swab for about 10 rounds or 10 seconds. Slowly remove the swab.
4) Immediately place the swab in the extraction tube containing extraction buffer or Viral transport media (VTM) tube.

6. Sample procedure

6.1. Direct nasopharyngeal swab

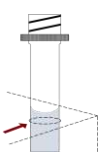
1) Collect nasopharyngeal swab following the specimen collection process.
2) Put the extraction tube containing sample-collected swab on the rack.
3) Rotate the swab against the side of the extraction tube 6-10 times, and allow the solution to mix for 1 min.
4) Before removing the swab, press both sides of the tube to squeeze the sample out of the soft head of the swab
5) Close the cap. Discard the swab in a biohazard container.

6.2. Sample collected in VTM

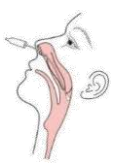
1) Insert the sample-containing VTM tube into the rack.
2) Pipette 300 µL of the VTM into a sample extraction buffer tube and mix gently.
3) Close the cap. Mix the solution gently and allow to stand for 1 min.

7. Assay procedure

1) Bring the test kit and the sample to the appropriate temperature (15-30 °C) prior testing.
2) Take the cassette out of the foil pouch. Place it on a clean, flat surface.
3) Open the top cap. Dispense 3 drops of the extracted sample solution (100 microliters) into the sample well, and do not move the cassette.
4) Wait until the colored band appears. Read the result within 15-20 min. Do not read after 30 min.

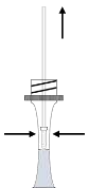


1. Uncap sample extraction tube and insert into the rack.

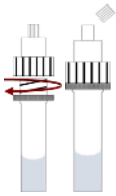


2.Collect nasopharyngeal swab.

3. Put swab into extraction tube and rotate 6-10 times. Let it stand for 1 min.



4. Remove the swab and squeeze the soft head against inner wall of the tube.



5. Close the cap and mix gently. Unscrew the top cap.

6. Dispense 3 drops into the sample well. Read result within 15-20 min.

8. Intended use

The K-Influenza A/B Antigen Test is a rapid, qualitative, colored chromatographic immunoassay. The test is intended for screening Influenza A and Influenza B infection by using nasopharyngeal swab specimens in the same cassette.

9. Precaution

1) Test should be performed by professional healthcare providers or trained operators only.
2) Do not eat, drink or smoke during sample collection and test performance.
3) Wear gloves during the test, and clean hands before and after performing the test.
4) Avoid spilling any specimen and wear a mask while performing the test.
5) Clean up the test area and equipment, using an appropriate disinfectant.
6) The tested device and contaminated materials should be disposed of, following standard regulations for biohazard material management.
7) Do not use if packaging is broken or pre-opened.
8) Do not use after expiration date.

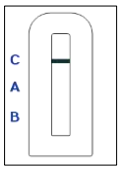
10. Warning

The K-Influenza A/B Antigen Test is a screening test. If the test result is positive, a nucleic acid test should always be performed further as a confirmatory test. If the result is negative, the patient's history, exposure period, time of infection, the presence of clinical signs and symptoms should also be considered for diagnosis.

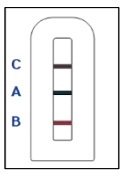
11. Quality control

Internal control: the K-Influenza A/B Antigen Test contains a control band or "C band". The C band develops after adding extracted specimen buffer. If the color of the control band ("C") is not visible after performing the test, the result is considered invalid. Repeating the test with a new device is recommended. If the same problem still occurs, stop using the test kit, and please contact the distributor.

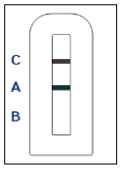
12. Result and interpretation



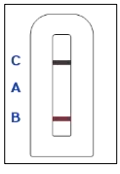
Negative for Influenza A and B: The presence of only one control line ("C" band).
Interpretation: No infection, or early infection of Influenza A or B. No Influenza A or B nucleocapsid antigens are detected.



Positive for Influenza A and B: The presence of three colored lines: the control "C" band, the "A" band, and "B" band.
Interpretation: Influenza A and B nucleocapsid antigens are detected.

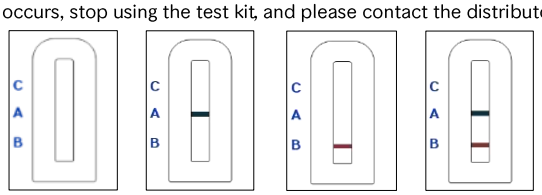


Positive for Influenza A: The presence of two colored lines: the control "C" band and the "A" band.
Interpretation: Influenza A nucleocapsid antigen is detected.



Positive for Influenza B: The presence of two colored lines: the control "C" band and the "B" band.
Interpretation: Influenza B nucleocapsid antigen is detected.

Invalid: The color of the control band ("C") is not visible, due to various reasons. Repeating the test with a new device is recommended. If the same problem still occurs, stop using the test kit, and please contact the distributor.



13. Test limitations

1) The test procedure and the result interpretation must be followed closely when using the test kit to detect Influenza A and B antigens in nasopharyngeal swab collected from suspected individuals. To ensure the most effective performance of the test, the process of specimen collection is critical.
2) The K-Influenza A/B Antigen Test is intended for professional use and aims for *in vitro* Influenza A and B diagnostic only.
3) The K-Influenza A/B Antigen Test is a qualitative method of detection. The intensity of the test 'A' and 'B' line does not correlate with the amount of virus in the specimen.
4) Sensitivity is different between Influenza viral strains due to .....รอความชัดเจนจาก version ภาษาไทย
5) Negative results indicate that there is no detectable amount of Influenza A and B nucleocapsid antigens present in the specimens. Negative results do not rule out Influenza A and B infection.
6) Negative results can occur when the concentration of Influenza A and B target antigens are lower than the limit of detection of the test, or no virus was collected on the specimen swab, or the epitope of target protein antigen was mutated and affected the affinity of the antibody utilized in the test.
7) Test is not intended to use for monitoring anti-viral therapy for Influenza A and B infection.

14. Clinical performance

1) Sensitivity and specificity

The K-Influenza A/B Antigen Test was evaluated in 172 nasopharyngeal swabs confirmed by real time RT-PCR. The performance of the K-Influenza A/B Antigen Test is shown in the table below.

RT-PCR	K-Influenza A/B Antigen Test			Sensitivity	Specificity
	Flu A	Flu B	Negative		
Flu A	27	0	1	96.4%	-
Flu B	0	23	1	95.8%	-
Negative	1	1	118	-	98.3%
Total	28	24	120	-	-

2) Hook effect

The K-Influenza A/B Antigen Test does not show inhibitory hook effect at target protein antigens of Influenza A and Influenza B at any given concentration.

3) Cross-reactivity

The cross-reactivity of the K-Influenza A/B Antigen Test was evaluated against pathogenic microorganisms and normal flora of the respiratory tract. Each type of organisms was tested in triplicate in the condition of presence and absence of inactivated Influenza A and Influenza B. No cross-reactivity was seen with the following microorganisms tested.

Potential Cross-reactant	Concentration	Cross-reactivity at A Test line	Cross-reactivity at B Test line
SARS-CoV-2 NP antigen	10 µg/mL	No (3/3 negative)	No (3/3 positive)
SARS-coronavirus NP antigen	10 µg/mL	No (3/3 negative)	No (3/3 positive)
MERS-coronavirus NP antigen	10 µg/mL	No (3/3 negative)	No (3/3 positive)
Human coronavirus 229E	10 µg/mL	No (3/3 negative)	No (3/3 positive)
Human coronavirus OC43	10 µg/mL	No (3/3 negative)	No (3/3 positive)
Human coronavirus NL63	10 µg/mL	No (3/3 negative)	No (3/3 positive)
Parainfluenza virus 1	180 µg/mL	No (3/3 negative)	No (3/3 positive)
Parainfluenza virus 2	180 µg/mL	No (3/3 negative)	No (3/3 positive)
Parainfluenza virus 3	180 µg/mL	No (3/3 negative)	No (3/3 positive)
Parainfluenza virus 4	180 µg/mL	No (3/3 negative)	No (3/3 positive)
Haemophilus influenzae	1.5 x 10^8 CFU/mL	No (3/3 negative)	No (3/3 positive)
Streptococcus pneumoniae	1.5 x 10^8 CFU/mL	No (3/3 negative)	No (3/3 positive)
Streptococcus pyogenes	1.5 x 10^8 CFU/mL	No (3/3 negative)	No (3/3 positive)
Candida albicans	10^6 CFU/mL	No (3/3 negative)	No (3/3 positive)
Pooled human nasal wash-representative of normal respiratory microbial flora	100%	No (3/3 negative)	No (3/3 positive)
Mycobacterium tuberculosis	>2×10^3 CFU/mL	No (3/3 negative)	No (3/3 positive)
Staphylococcus aureus	1.5 x 10^8 CFU/mL	No (3/3 negative)	No (3/3 positive)
Staphylococcus epidermidis	1.5 x 10^8 CFU/mL	No (3/3 negative)	No (3/3 positive)

4) Chemical interference

Tested chemical substances are naturally present in respiratory specimens or artificial substances introduced into the nasal cavity or nasopharynx. The interference substances were used to evaluate the K-Influenza A/B Antigen Test at the concentration listed in the table below. All of the tested substances were found to have no effect on test performance.

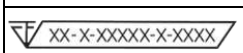
Interfering Substance	Concentration	Interference (Yes/No)
Whole Blood	4%	No (5/5 negative)
Mucin	0.5%	No (5/5 negative)
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL	No (5/5 negative)
CVS Nasal Drops (Phenylephrine)	15% v/v	No (5/5 negative)
Afrin (Oxymetazoline)	15% v/v	No (5/5 negative)
CVS Nasal Spray (Cromolyn)	15% v/v	No (5/5 negative)
Zicam	5% v/v	No (5/5 negative)
Sore Throat Phenol Spray	15% v/v	No (5/5 negative)
Mupirocin	10 mg/mL	No (5/5 negative)
Fluticasone Propionate	5% v/v	No (5/5 negative)
Tamiflu (Oseltamivir Phosphate)	1.5 mg/mL	No (5/5 negative)

15. Limit of detection

The limit of detection (LoD) of K-Influenza A/B Antigen Test was evaluated with nucleocapsid antigens of Influenza A (H1N1) and Influenza B by making serial dilution of confirmed specimens. The test was repeated 20 times for each sample. The result is as follows.

Virus Strains	Dilution factor	Concentration (HA Units/mL); LoD	Positive / Total
Influenza A (H1N1)	X1/1024	19.53	20/20
Influenza B	X1/1024	23.11	20/20

Symbols and abbreviations

Symbol	Meaning
	One time use only, do not reuse the test
	Store at temperature between 1°C - 30°C
	*in vitro* diagnostic use only
	Consult instructions for use
	Manufacturer
	Registered number. FDA approved.
Lot No.	Batch code
MFD	Manufacturing date
EXP	Expiration date

KBIOSCIENCES
Manufactured by
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