AMAZING BIO™

COVID-19 Ag/Ab(IgG/IgM) Sealing Tube Twin Test Strip (Colloidal Gold)

Instructions for Use

CE IVD REF

In Vitro Diagnosis
For Professional Use

AB00301

INTENDED USE

Amazingbio COVID-19 Ag/Ab(IgG/IgM) Sealing Tube Twin Test Strip is for the qualitative detection of SARS-associated corona virus (SARS-CoV-2) antigens from human oropharyngeal, nasal and saliva, antibodies (IgM/IgG) from human whole blood (sodium citrate, sodium heparin, dipotassium EDTA or fingerstick whole blood) to assist in the diagnosis of Covid-19 infection. This unique design combines test strip and diluent together in one tube providing a concept: Sampling +upending = test. It also seals the clinical sample and swab in the tube to avoid pathogen contamination and potential biosecurity substances (such as virus from nasal swab sample) spreading to the operator and the environment. It is intended for use by medical professionals, trained clinical laboratory operators or individuals trained in point of care settings, it's also good for use of self-test and home user.

PRINCIPLE

The COVID-19 Ag/Ab(IgG/IgM) Sealing Tube Twin Test Strip has two test strips, one is the COVID-19 antigen test strip and the other is the COVID-19 antibody test strip.

The COVID-19 Ag/Ab(IgG/IgM) Sealing Tube Twin Test Strip is based on a sandwich method immunochromatographic assay for the highly sensitive detection of COVID-19 nucleoprotein in human oropharyngeal, nasal and saliva swabs, and COVID-19 IgG or IgM in human whole blood providing with invisible test area (T) and control area (C). When a sample is released into the tube buffer, the liquid laterally migrate along the test strip. If sufficient COVID-19 antigens are present in the sample, a visible line of test area (T) appears. If sufficient COVID-19 IgG or/and IgM in the sample of test area IgG/T1 or/and IgM/T2 will appears. As process control, a colour band shall appear in the control area (C) to confirm that sufficient sample has been absorbed. The test result is visually interpreted after 15 minutes basing on the presence or absence of visually recognizable colored lines. The strip signals could last several days for double check if necessary.

ADDITIONAL REQUIRED MATERIAL (not included in the test kit)

A Stopwatch or timer

REAGENTS AND MATERIALS PROVIDED

1. Sealing tube test strip with sealed diluent
2. Sample swab
3. Biood lancet and alcohol bag

PACKING SPECIFICATION

20 kits X 1 pouch /box
2 kits X 1 pouch/box
1 kit X 1 pouch /box

STORAGE

The kit can be stored at room temperature (4-30°C). It should be protected from direct sunlight, humidity and heat until the expiry date (18 months) printed on the foil pouch. Please seal the pouch carefully if the batch tube is still left inside and you should use up the kit within one week once the pouch is opened. Care must be taken to protect the kit components against pathogen contamination. DO NOT FREEZE.

RESTRICTIONS

1. The test can be used for the qualitative detection of SARS-CoV-2 antigens in the oropharyngeal, nasal and saliva samples, and of COVID-19 IgG/IgM in human whole blood samples. The exact concentration of SARS-CoV-2 antigens and COVID-19 IgG/IgM cannot be determined in this test.
2. If the viral load of the sample is below the detection limit of the test, the test may result in a negative result.
3. A negative result does not exclude viral infection other than SARS-CoV-2 and should be confirmed by molecular diagnostic methods if COVID-19 is suspected.
4. As with all diagnostic tests, a final clinical diagnosis should not be based on the result of a single test, but should be submitted by the physician after evaluation of all clinical results and laboratory findings.
5. The SARS-CoV-2 antigen and COVID-19 IgG/IgM rapid test can detect both viable and non-viable SARS-CoV-2 material. The performance of the SARS-CoV-2 rapid test depends on the viral load and may not correlate with other diagnostic methods performed on the same sample.
6. A positive result does not preclude co-infection with other pathogens.

PRECAUTIONS

1. Do not reuse the kit.
2. Do not use the kit after the expiry date.
3. Do not use if the foil pouch is damaged or open.
4. Do not return the swab to the kit package after use.
5. Do not mix components from kits with different batch numbers.
6. Avoid microbial contamination of the reagents.
7. The kit should be used within half an hour after the opening of the foil pouch to prevent detection failure due to moisture absorption.

SAMPLE PREPARATION NOTE

(1) Oropharyngeal Swab Sample

a) Remove a oropharyngeal swab from the package.
b) Place the swab into the patient's mouth until it reaches the posterior; keep insert until resistance is encountered or the distance
c) Slowly and gently make one rotation of the swab over the surface of the posterior oropharyngeal (to prevent reflex cough, stop for one minute).
d) Slowly remove the swab from the nostril.

(2) Nasal Swab Sample

a) Remove a nasal swab from the package.
b) Place the swab into the patient's nostrils until it reaches the turbinates (less than one inch into the nostril).
c) Gently rotate 5 times the swab or more over the surface of the nasal.
d) Slowly remove the swab from the nostril while rotating it.
e) Repeat sample collection using the same swab in another nostril.

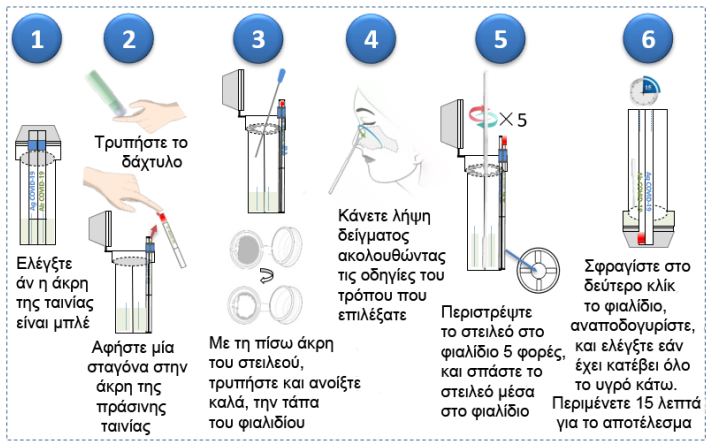
(3) Saliva Swab Sample

a) Remove a saliva swab from the package.
b) Place the swab into the patient's mouth as comfortable and and rub along the lower gums (see close up image) in a back and forth motion. Gently rub the gums 10 times. If possible, avoid rubbing the teeth.
c) Gently repeat rubbing motion on the opposite side of the mouth along the lower gums for an additional 10 times.
d) Slowly remove the swab from the mouth.

(4) Whole Blood specimens

Using a lancet to collect Fingerstick Whole Blood specimens.
a) Gently massage the inner side of the left ring finger to make the local tissues congested naturally.
b) Wipe the blood collection area with a 75% ethanol cotton ball and dry it.
c) Use the thumb and index finger of the left hand to fix the blood collection site to tighten the skin and subcutaneous tissue and pierce the sterile lancet with the right hand from the ventral inner side of the finger to a depth of 2-3mm and take out the lancet immediately. One lancet one person to avoid cross infection.
d) Wipe away the first drop of blood.
e) Gently rub the hand from wrist to finger to form a rounded drop of blood over the puncture site.

TEST PROCEDURE



a) Use a micropipette or dropper to take 10μL whole blood sample, directly onto the strip tip which is out of the tube, or put the strip tip close to the finger blood and let the blood go to the marked line.
b) Take out the kit and equilibrate to room temperature (20~25°C).
c) Open the foil pouch, take needed sealing tube test strips for test and seal the pouch carefully if batch tube is still left inside.
d) Please check the paper on the tip of the test strip and discard the tube if it turns white or pink. Proceed to the next step if the paper remains blue.
e) Take out the swab from the bag and use the end of the swab pole to break the aluminum foil before samples collection. Make sure the hole is big enough.
f) Follow the above instructions **(sample preparation note)** depending on the desired sampling (saliva, nasal, oropharyngeal).

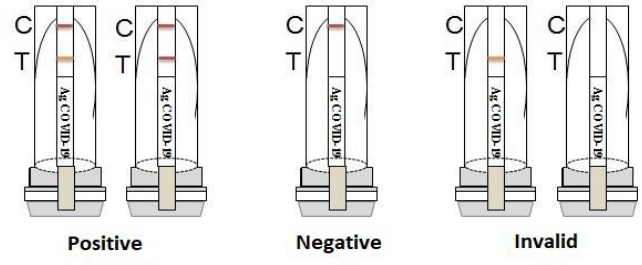
g) Insert sampled swab into tube, bend soft swab to touch the inner wall (or insert it to a center hole surrounding by special inner walls), Rotate it left and right 5 times while make sure it touching tube inner wall all the way to release sample into buffer. Switch swab up and down 3 times. Finally break its pole and leave its tip in the tube.

h) Press the cap firmly to close tube and make sure 2 clicks have been heard in total, otherwise Press again to the bottom level. Turn the tube upside down and gently knock the cap on the table once, ensure all the liquid reaches the tube cap and fully contact with the strip. If the liquid does not fully contact the strip, gently knock the tube cap on the table to ensure that all the liquid reaches the tube cap and fully contacts the strip. The buffer will migrate along the strip and the liquid will rise up the white membrane.

i) Interpret the result after 15 minutes. (The strip in this creative sealing tube can avoid signal changing since there is no back migration in the strip).

INTERPRETATION OF RESULT

1**. COVID-19 Antigen:**



POSITIVE (+)

The presence of both C band and T band, no matter T band is clear or vague. The result is SARS-CoV-2 positive.

NOTE: The color intensity of the band in the test area (T) may vary depending on the concentration of SARS-CoV-2 antigens in the sample. Therefore, any colored or shaded band in the test area (T) should be considered as a positive result.

MEASURES: There is a suspicion of COVID-19 infection, contact your GP or local health department immediately. Follow local self-isolation guidelines and have a PCR test performed to confirm the test result.

NEGATIVE (-)

Only clear C band appears.

MEASURES: A negative test result does not rule out the presence of SARS-CoV-2 viruses in general and is always only a snapshot. Therefore, even if you have a negative result, continue to observe all contact rules and observe the protective measures. In case of suspicion, repeat the test after 1-2 days.

INVALID

No colored band appears in C zone, no matter whether T band appears. It is necessary to retest with a new tube strip.

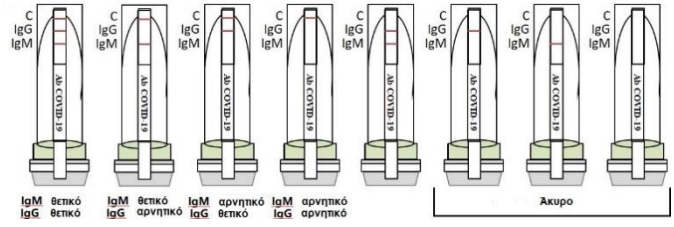
NOTE: Insufficient sample volume, faulty test procedure or a failed test are the most common reasons for an invalid result.

MEASURES: Repeat test. If the result of the repeated test is also invalid, contact a doctor or COVID-19 test center.

QUALITY

The C-line is an internal process control. When the test runs and the reagents work, this line is always displayed. External controls are not included in the kit.

2**. COVID-19 Antibody**



POSITIVE (+)

1. The presence of zone C, IgG / T1 and IgM / T2, means that COVID-19 IgG / T1 and IgM / T2 were detected (**possible secondary SARS-CoV-2 infection).**

2. The presence of C band and IgG / T1, no matter IgG / T1 band is clear or vague, which means that COVID-19 IgG / T1 was detected **(antibodies detected after disease or vaccination).**

3. The presence of C band and IgM/T2, no matter IgM/T2 band is clear or vague, which means that COVID-19 IgM/T2 was detected **(indication of primary infection by SARS-CoV-2).**

NEGATIVE (-)

Only clear C band appears, which means no COVID-19 IgG/T1 or IgM/T2 was detected **(no disease or antibodies were detected).**

INVALID

No colored band appears in C zone, no matter whether other band appears. which means no COVID-19 IgG/T1 or IgM/T2 was detected. It is necessary to retest with a new test strip.

CONSISTENCY ANALYSIS OF TEST RESULTS

Consistency comparison of experimental reagent and reference reagent

In this study, 500 nasal and 500 saliva and 500 oropharynx swab samples were obtained in the clinical performance study to compare the results of the COVID-19 Antigen Sealing Tube Test Strip and the nucleic acid results. Analyzed the clinical performance data of the COVID-19 test results, and tested 200 samples that were positive for nucleic acid through the COVID-19 antigen sealing tube test strip, and 197 were positive. There were 300 negative samples for nucleic acid in the experimental reagents, and 297 negative samples were tested by the Reagent. Hence, the sensitivity and specificity were 98.5% and 99% respectively.

Table 1 Overall Clinical Study Results for Nasal Swab

Reagent test results	**PCR Comparator**	
	positive	Negative
positive	197	-
negative	3	-
Subtotal	200	-
Reagent test results	PCR Comparator	
	positive	Negative
positive	-	3
negative	-	297
Subtotal	-	300

Positive Percent Agreement (PPA）= 98.5%（95.3%~99.7%）
Negative Percent Agreement (NPA）= 99.0%（96.9%~99.7%）
Accuracy = 98.8%

Table 2 Overall Clinical Study Results for Saliva Swab

Reagent test results	**PCR Comparator**	
	positive	Negative
positive	197	-
negative	3	-
Subtotal	200	-
Reagent test results	PCR Comparator	
	positive	Negative
positive	-	3
negative	-	297
Subtotal	-	300

Positive Percent Agreement (PPA）= 98.5%（95.3%~99.7%）
Negative Percent Agreement (NPA）= 99.0%（96.9%~99.7%）
Accuracy = 98.8%

Table 3 Overall Clinical Study Results for Oropharynx Swab

Reagent test results	**PCR Comparator**	
	positive	Negative
positive	197	-
negative	3	-
Subtotal	200	-
Reagent test results	PCR Comparator	
	positive	Negative
positive	-	3
negative	-	297
Subtotal	-	300

Positive Percent Agreement (PPA）= 98.5%（95.3%~99.7%）
Negative Percent Agreement (NPA）= 99.0%（96.9%~99.7%）
Accuracy = 98.8%

Table 4 The COVID-19 antibody was conducted in prospective studies with serum samples, Sensitivity, specificity and correlation as follows:

COVID-19 IgM		ELISA		Total
		Positive	Negative	
COVID-19 Ag/Ab(IgG/IgM) Sealing Tube Test Strip	Positive	108	2	110
	Negative	2	101	103
Total		110	103	213
COVID-19 IgG		ELISA		Total
		Positive	Negative	
COVID-19 Ag/Ab(IgG/IgM) Sealing Tube Test Strip	Positive	104	2	106
	Negative	2	106	108
Total		106	108	214

Relative sensitivity: 98% (95% CI: 93% - 99%)
Relative specificity: 98% (95% CI: 93% - 99%)
Relative accuracy: 98% (95% CI: 97% - 100%)

CROSS REACTIVITY (ANALYTICAL SPECIFICITY) STUDY:

Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) Cross Reactivity

The potential cross-reactivity of the Amazingbio COVID-19 antigen sealing tube test strip (Colloidal Gold) was evaluated by testing twenty-seven (27) commensal and pathogenic microorganisms (Wet testing: 16 viruses, 9 bacteria, 1 yeast, and 1 pooled human nasal wash) that may be present in the nasal cavity. Each organism, virus, bacteria, and yeast were tested in triplicate (3) in the absence or presence of 3x LoD of the heat-inactivated SARS-CoV-2 virus (NR-52287). No cross reactivity or interference was seen with the following microorganisms when tested at the concentration presented in the table below.

Table 1 Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) Cross Reactivity

Virus/Bacteria/Parasite name	Strain	Wet-testing Concentration/ *In silico* testing	Cross Reactivity Results (Number of Positive/Total)
Human coronavirus	229E	$1.6 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Human coronavirus	OC43	$1.6 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Human coronavirus	NL63	$1.6 \times 10^{3.0}$ TCID50/mL	No Cross-Reactivity (0/3)
MERS-coronavirus	EMC/2012	$1.6 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Adenovirus	Serotype 5	$1.6 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Human metapneumovirus (hMPV)	TN/91-320	$1.6 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Human parainfluenza virus 1	HPIV1/FRA/29221106/2009	$8.9 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Human parainfluenza virus 2	Greer	$1.0 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Human parainfluenza virus 3	NIH 47885	$1.6 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Human parainfluenza virus 4a	M-25	$1.6 \times 10^{3.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Human parainfluenza virus 4b	19503	$5.0 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Influenza A	A/California/07/2009	$5.2 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Influenza B	B/Hong Kong/330/2001 (Victoria Lineage)	$1.8 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Enterovirus	71/Tainan/4643/98	$1.6 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Respiratory Syncytial Virus A	1998/12-21	$2.8 \times 10^{5.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Rhinovirus	16	$>5.0 \times 10^{3.0}$ TCID50/mL	No Cross-Reactivity (0/3)
Haemophilus influenzae	Type B CK	>104.0 cfu/vial*	No Cross-Reactivity (0/3)
Streptococcus pneumoniae	Z022	$3.6 \times 10^{6.0}$ cfu/mL	No Cross-Reactivity (0/3)
Streptococcus pyogenes	Z018/M58	$3.9 \times 10^{6.0}$ org/mL	No Cross-Reactivity (0/3)
Pooled human nasal wash	N/A	N/A	No Cross-Reactivity (0/3)
Bordetella pertussis	18323 [NCTC 10739]	$4.8 \times 10^{6.0}$ cells/mL	No Cross-Reactivity (0/3)
Mycoplasma pneumoniae	M129	$2.7 \times 10^{6.0}$ cfu/mL	No Cross-Reactivity (0/3)
Chlamydia pneumoniae	TW-183	$9.1 \times 10^{6.0}$ IFU/mL	No Cross-Reactivity (0/3)
Legionella pnuemophila	Philadelphia	$1.9 \times 10^{6.0}$ cfu/mL	No Cross-Reactivity (0/3)
Staphylococcus aureus	MRSA	$6.5 \times 10^{5.0}$ cfu/mL	No Cross-Reactivity (0/3)
Staphylococcus epidermidis	MSSE;HER 1292	$7.7 \times 10^{6.0}$ cfu/mL	No Cross-Reactivity (0/3)
Candida albicans	Y537	$5.0 \times 10^{6.0}$ cfu/mL	No Cross-Reactivity (0/3)

* No quantitation data provided. The stock concentration on the Certificate of Analysis (CoA) was $>10^4$ cfu/vial.
** IFU/mL is Infectious Units per milliliter.

Table 2 Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) organisms interference

Virus/Bacteria/Parasite name	Strain	Wet-testing Concentration	Interference Results (Number of Positive/Total)
Human coronavirus	229E	$1.6 \times 10^{5.0}$ TCID50/mL	No Interference (3/3)
Human coronavirus	OC43	$1.6 \times 10^{5.0}$ TCID50/mL	No Interference (3/3)
Human coronavirus	NL63	$1.6 \times 10^{3.0}$ TCID50/mL	No Interference (3/3)
MERS-coronavirus	EMC/2012	$1.6 \times 10^{5.0}$ TCID50/mL	No Interference (3/3)
Adenovirus	Serotype 5	$1.6 \times 10^{5.0}$ TCID50/mL	No Interference (3/3)
Human metapneumovirus (hMPV)	TN/91-320	$1.6 \times 10^{5.0}$ TCID50/mL	No Interference (3/3)
Human parainfluenza virus 1	HPIV1/FRA/29221106/2009	$8.9 \times 10^{5.0}$ TCID50/mL	No Interference (3/3)
Human parainfluenza virus 2	Greer	$1.0 \times 10^{5.0}$ TCID50/mL	No Interference (3/3)
Human parainfluenza virus 3	NIH 47885	$1.6 \times 10^{5.0}$ TCID50/mL	No Interference (3/3)
Human parainfluenza virus 4a	M-25	$1.6 \times 10^{3.0}$ TCID50/mL	No Interference (3/3)
Human parainfluenza virus 4b	19503	$5.0 \times 10^{5.0}$ TCID50/mL	No Interference (3/3)
Influenza A	A/California/07/2009	$5.2 \times 10^{5.0}$ TCID50/mL	No Interference (3/3)

ENDOGENOUS INTERFERENCE SUBSTANCES STUDIES:

Endogenous interference substances studies of the Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) were evaluated by testing the following substances listed in the table below. Each substance was tested in triplicate (3) with 3x LoD, 2250 $TCID_{50}$/mL, inactivated SARS-CoV-2 virus (NR-52287). All samples tested produced expected results, demonstrating that the Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) performance was not affected by any of the 14 potentially interfering substances listed in the table 5 below.

Table 3 Endogenous interference study of Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold)

Potential Interfering Substances	Active Ingredient	Test concentration	Results (Number of Positive/Total)
Whole Blood	Blood (human)	4 % V/V	No Interference (3/3)
Mucin	Mucin protein, Type I-S	0.5 % W/V	No Interference (3/3)
Chloraseptic	Benzocaine, Menthol	0.15 % W/V (1.5mg/ml)	No Interference (3/3)
Naso Gel (NeiMed)	Saline	5.0 % V/V	No Interference (3/3)

Nasal Spray	Phenylephrine	15.0 % V/V	No Interference (3/3)
Afrin	Oxymetazoline	15.0 % V/V	No Interference (3/3)
Zicam	Oxymetazoline, Hydrochloride	5.0 % V/V	No Interference (3/3)
Nasal Spray (Cromolyn)	Cromolyn sodium	15.0 % V/V	No Interference (3/3)
Alkalol	Galphimia glauca, Luffa operculata, Sabadilla	1:10 dilution	No Interference (3/3)
Sore Throat Phenol Spray	Phenol	15.0 % V/V	No Interference (3/3)
Tobramycin	Tobramycin	0.0004% W/V (4ug/ml)	No Interference (3/3)
Mupirocin	Mupirocin	1.0 % W/V (10mg/ml)	No Interference (3/3)
Fluticasone Propionate	Fluticasone propionate (glucocorticoid)	5.0 % V/V	No Interference (3/3)
Tamiflu	Oseltamivir	0.5 % W/V (5 mg/mL)	No Interference (3/3)

HIGH-DOSE HOOK EFFECT:

The high-dose hook effect of the Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) was evaluated by testing the following serial dilutions of the characterized SARS-CoV-2 listed in the table. No high dose hook effect was observed when tested with up to a concentration of 1.5 x $10^{5.0}$ or 1.0 x $10^{5.0}$ $TCID_{50}$/mL of the inactivated SARS-CoV-2 virus (NR-52287) to the Hercin 2019-nCoV Antigen Test Kit.

Table 4 High-dose hook effect of Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold)

SARS-CoV-2 Concentration (TCID50/mL)	High-dose Hook Effect Test Results (Number of Positive/Total)
$1.5\times10^{5.0}$	Not Identified (3/3)
$1.0\times10^{5.0}$	Not Identified (3/3)

【INDEX OF SYMBOLS】

IVD	For in vitro diagnostic use only.		Do not reuse.
	Expiry date.		See instruction for use.
	Warning, please refer to the instructions in the annex.		Manufacturer.
4℃ 30℃	Temperature scope within which the product is reserved.	LOT	Batch number.
REF	Catalog #	Σ	Tests per kit.
EC REP	European union authorized representative.		Keep dry.
	Keep away from sunlight.		Don't use the product when the package is damaged.
	Biological risks.		Date of manufacture.
CE	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC.		

Manufacturer:
Amazing Biotech (Shanghai) Co.,LTD.
3/F, Building 1, No. 48-52 Gucui Road, Xinchang Town, Pudong New Area, Shanghai, China.
E-mail: int_sales@amazingbio.cn Tel: +86(0)21-68071355

European Representative:
Lotus NL B.V.
Koningin Julianaplein 10, 1e Verd, 2595AA, The Hague, Netherlands.
E-mail: peter@lotusnl.com Tel: +31644168999

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