# A chicken IgY can efficiently inhibit the entry and replication of SARS-CoV-2 by targeting the ACE2 binding domain in vitro

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**Graphical Abstract** (see Fig.1.)

## Abstract

COVID-19 pneumonia is spreading widely in the world now. Currently, no specific antiviral drugs have been developed. The vaccine is the most effective way to control the epidemic. Passive immune antibodies are also an effective method to prevent and cure COVID-19 pneumonia. We used SARS-CoV-2 S-RBD as an antigen to immunize layers in order to extract, separate and purify SARS-CoV-2-IgY from egg yolk. SARS-CoV-2-IgY(S-IgY) can block the entry of SARS-CoV-2 into the Cells and reduce the viral load in cells. The $EC_{50}$ of W3-IgY (S-IgY in the third week after immunization) is1.35 ± 0.15nM. The $EC_{50}$ of W9-IgY (S-IgYin the ninth week after immunization) is 2.76 ± 1.54 nM. When the dose of S-IgY is 55 nM, the fluorescence representing intracellular viral protein is obviously weakenedin Immunofluorescence microscopy.

Results of Sars-CoV-2 /VeroE6 cell experiment confirmed that S-IgY had strong antiviral effecton SARS-Co-V-2, and its $EC_{50}$ is 27.78 ± 1.54nM vs 3259 ± 159.62 nM of Redesivir (differ›106 times，$P < 0.001$ ).

S-IgY can inhibit the entry and replication of SARS-CoV-2, which is related to its targeting the ACE2 binding domain.

S-IgY is safe, efficient, stable and easy to obtain. This antibody may have the potential to be an effective method for the prevention and treatment of COVID-19 pneumonia.

**Keywords: SARS-CoV-2; COVID-19; SARS-CoV-2-IgY(S-IgY); Spike protein; ACE2 binding domain**

## Introduction

The 2019 novel coronavirus (SARS-CoV-2) is a new coronavirus strain found in the human body in 2019. During the incubation period, the virus is still contagius. The main transmission ways of the coronavirus are air transmission and contact, and the intermediate host is still unclear (1).Globally, on 13 February 2021, there have been more than107.68 million confirmed cases of COVID-19, including about 2.38 million relative deaths. As a global "grey rhinoceros" and "black swan event", the COVID-19 pandemic impacts not only human health, but also the world's economy and politics. The severity of the impacts depends on three aspects: the exact time when the pandemic began to spread, the strength of the governmental epidemic prevention and the measures used to control the epidemic in various countries (source: WHO) (2). By modeling and analyzing factors such as seasons, mode of transmission, cross-immunity between immunity and coronavirus, it is predicted that the COVID-19 pandemic may last until 2024 (3).

SARS-CoV-2 can be divided into three categories: A, B and C. Type A is the root of the virus outbreak, which is concentrated in infected people in the United States and Australia. B strain is mutated from A, which mainly appears in China (i.e. Wuhan). Type C evolves from type B, which is mainly carried by infected people in Europe (4). At present, many SARS-CoV-2 mutants are spreading in

Britain, France, India, Denmark, Japan, Portugal, Norway, Jordan, South Korea and other places, which makes SARS-CoV-2 more contagious and more difficult to prevent and control than before(source: WHO)(5).

Spike protein S, envelope E, membrane glycoprotein M and nucleocapsid N are the four essential coding structural proteins for coronavirus to complete virus assembly and infection. It is found that the S protein, which is the fusion protein of the virus, is located on the surface of the SARS-CoV-2. The S protein is responsible for the combination of the virus and the host cell receptor, which mediates the first step of SARS-CoV-2 infection (6). Each trimeric S protein monomer is about 180 KD and contains two subunits, S1 and S2, mediating attachment and membrane fusion, respectively. In the structure, N- and C- terminal portions of S1 fold as two independent domains, N-terminal domain (NTD) and C-terminal domain(C-domain). Either NTD or C-domain can serve as the receptor-binding domain depending on the type of virus (7,8). The receptor of SARS-CoV-2 invading human cells is angiotensin converting enzyme 2(ACE2) (9).

The primary pathological process of COVID-19 patients is the inflammatory reaction caused by the SARS-CoV-2 virus combined with ACE2. Fever, dry cough and fatigue are common symptoms of COVID-19. Most patients have ground glass opacity and lymphocyte reduction in chest CT scan. Patients with underlying medical conditions and the elderly are more likely to suffer from severe illness and death after infection(10,11).

The results of a large-scale randomized controlled trial (RCT) of glucocorticoid in the treatment of COVID-19 pneumonia show that short-term oral or intravenous administration of low-dose hormone can greatly reduce the mortality of patients who need oxygen inhalation or non-invasive ventilator within 28 days (12). Remdesivir was approved by the U.S FDA as the first COVID-19 treatment drug in the United States. Remdesivir might shorten the time to clinical improvement among hospitalized adults with severe COVID-19 (13), but it is not recommended as a first-line treatment by the World Health Organization (14). Since the COVID-19 outbreak, countries around the world have accelerated the development of the COVID-19 vaccine. By December 2020, 60 candidate COVID-19 vaccines have been approved for clinical trials, and 7 of these vaccines (3 inactivated vaccines, 2 mRNA nucleic acid vaccines and 2 vector vaccines) have been approved for emergency use or conditional marketing (source: WHO) (15,16). The effective rate of mRNA vaccine of Pfizer pharmaceutical Co., Ltd. inthe United States is 95%. The production and persistence of immune neutralizing antibodies in vaccinated people need to be confirmed (17).

Antibodies that can effectively neutralize SARS-CoV-2 were found in RBD specific monoclonal antibodies from B lymphocytes of patients. This process is related to the competition of ACE2 binding RBD (18). Using recombinant human ACE2 antibody protein purified in vitro to "neutralize" SARS-CoV-2 virus can reduce its infection ability by 1000-5000 times (19). The mechanism of plasma therapy is to use the specific antibody found in the recovered patients' plasma to

quickly identify and capture the virus and activate the complement system in the body to eliminate the virus. Plasma therapy has achieved good results in major epidemics, such as SARS, MERS, Ebola and H1N1 influenza, which were common epidemic in recent years (20).Recent research data showed that the transfusion of the plasma that contains high titer neutralizing antibodies from the SARS-CoV-2 infected patients in convalescence to the critically ill patients can restore the organ functioning within a short time, give a negative result in virus nucleic acid detection and increase specific antibody concentration (21). However, because of the shortage of plasma in convalescence, the disparity between plasma demand and supply and the safety of donor plasma, it is still not feasible to obtain qualified plasma in convalescence.

Egg yolk immunoglobulin Immunoglobulin Y (IgY) extracted from immunized poultry egg yolk is an excellent source of antibody for passive immunization (22). IgY has long been used to prevent and treat infectious diseases of poultry and livestock (23).The research on IgY in the diagnosis and treatment of human diseases is becoming increasingly popular.Many instances indicate this tendency,like Anti-Vibriocholerae IgY (24), Antibodies (IgY) for Prophylaxis and Treatment of Rotavirus Diarrhea in Human and Animal Neonates (25),like anti-vibriocholerae IgY (24), antibodies (IgY) for prophylaxis and treatment of rotavirus diarrhea in human and animal neonates (25), Neutralization antibodies against highly pathogenic H5N1, H1N1 virus, avian influenza virus(26,27), IgYof SARS virus (28), and hand-foot-and-mouth disease (29). In this essay, we are

going to report a study of an egg antibody ( SARS-CoV-2-IgY ) with high neutralization ability to SARS-CoV-2 in vitro.

## Results

### 1. The expression and purification of SARS-CoV-2 S-RBD

SDS-PAGE results showed that the fusion protein of SARS-CoV-2 S-RBD was between 70kD and 55kD, in according with the predicted molecular weight 66.2KD was made by the pCMV-DsbC-RBD fusion expression vector (provided by Sina Biological Company) and band one and two showed the purity of the protein was very high ( Fig.2.).

### 2. The preparation of total IgY

The laying hens were immunized with antigen of SARS-CoV-2 S-RBD for 4 times, we obtained total S-IgY against SARS-CoV-2 from egg yolk by ways of water extraction, salting out, dialysis and ultrafiltration, etc. Results of the gel electrophoresis SDS-PAGE showed that the heavy chain band was at 70kD and the light chain band was at 25kD, and the total IgY purity was about 85% (Fig.3.).

### 3. The specificity of S-IgY

In order to confirm the recognition and binding ability of SARS-CoV-2 to S-RBD antigen, Western blotwere done. The dilution of primary antibody (S-IgY) was 1: 10000, and the dilution of secondary antibody (HRP-labeled goat

anti-chicken) was 1: 10000. The results showed that SARS-CoV-2 had a good effect on S-RBD antigen The antibody band was clearly located between 55 KD and 70KD, which was consistent with the electrophoresis position of S-RBD antigen(66.2KD), and there were few heterobands, which indicated that the content of nonspecific IgY in total IgY was less, and the proportion of specific S-IgY was higher. (Fig.4.).

**4. S-IgY blocks cell entry of SARS-CoV-2**

For investigating the role of S-IgY in neutralizing SARS-CoV-2 and preventing SARS-CoV-2 from entering target cells, Pseudotyped VSV luciferase-reporter particlesbearing SARS-CoV-2 spike (S) protein (pSARS-CoV-2) were used to reflect the virus entry activitywas done. The resultsof viral load in the cells detected by realtime qPCR demonstrated thatS-IgY can efficiently blocking the cell entry of virus. The$EC_{50}$ of W3-IgY is 1.35 ± 0.15 nM and $EC_{50}$of W9-IgYis 2.76±1.54 nM (Fig.5.).

**5.S-IgYreduce the replication of SARS-CoV-2**

**5.1 Immunofluorescence**

Cell Immunofluorescence experiment was done to detect the SARS-CoV-2-S protein expression in Vero E6 cells,the results showed that that S-IgY could effectively inhibit the expression of SARS-CoV-2-S protein.

SARS-CoV-2-S proteinwere observed in the pre-immunizedIgY (Pre-IgY) group.When the concentration of S-2-IgY was 55nM, the SARS-CoV-2-S protein fluorescence in the field of vision was significantly reduced. About 50% of the fluorescence was suppressed; as the concentration of S-IgY was 110nM, the fluorescence disappeared completely in the field of vision. The SARS-CoV-2was completely suppressed by S-IgY (Fig.6.).

### 5.2 qRT-PCR

SARS-CoV-2 (100PFU) was incubated with an equal volume of Pre-IgY, S-2-IgY and Remdesivir in different concentration gradients in VeroE6 cells. The results showed that different doses of S-IgY had an obvious inhibitory effect on the cellular SARS-CoV-2 content (copy number) with $EC_{50}$ of 27.78 ± 1.54 nM. Remdesivir's $EC_{50}$ against SARS-CoV-2 was 3259 ± 159.62 nM,

The inhibitory effect of S-IgY on SARS-Cov-2 replication was 106 times stronger more than that of Remdesivir In vitro (Fig.7.).

## Discussion

Since the outbreak of infectious diseases of SARS-CoV-2 in early 2019, COVID-19 virus has quickly spread worldwide. Currently, the World Health Organization believes that no specific drug can produce antiviral effects except the use of glucocorticoid for severe patients. The main hope of controlling the

COVID-19 pandemic is placed on the use of vaccines. However, the vaccine also has some problems, such as vaccine failed due to unsuitability for people, short supply, individual differences in immune response, antibody titer and retention time, etc. Although keeping social distance including the use of face masks is an effective way to prevent infection, common surgical masks cannot completely block the air transmission of SARS-CoV-2 (30). Further, preventive medicine and external disinfectants cannot be used in real-time. To sum up, it is very necessary to develop new control methods for SARS-CoV-2.

There is convincing scientific evidence for the prevention and treatment of passive immune specific antibodies in SARS-CoV-2. However, human antibodies are expensive and need to bestrict cryopreservation which are not suitable as alarge-scale popularization and application.

We used SARS-CoV-2 S-RBD as an antigen to immunize hens to obtain SARS-CoV-2-IgY. Our study results confirmed that this antibody could recognize SARS-CoV-2 S-RBD antigen and specifically bind with it, thus blocking the interaction between S protein and ACE2 and preventing infection caused by the interaction between S protein and ACE2. The results of Real-time quantitativeRT-PCR（qRT-PCR）and the immunofluorescence experiment have proved that S-IgY can prevent SARS-CoV-2 from entering human cells.

Resuts of SARS-CoV-2 /VeroE6 cell experiment confirmed that S-IgY has strong antiviral effecton Sars-Co-V-2, and its $EC_{50}$was 27.78±1.54nM vs 3259±159.62 nM of Redesivir (differ > 106 times，$P<0.001$ ).

S-IgY can not only block SARS-CoV-2 from entering target cells, but also effectively inhibit replication of SARS-CoV-2 in cells.

For critically ill patients with pneumonia in COVID-19, the inflammatory storm caused by antibody therapy is also a problem worthy of great attention

From a study among patients in SARS-CoV-2, Wuhan, China in 2019, ICU patients had higher plasma levels of IL-2, IL-7, IL-10, GSCF, IP10, MCP1, MIP1A, and TNFα compared to non-ICU patients (31). Hypersensitivity inflammatory storm caused by virus infection is considered to be associated with the activation of complement C5a (32). IgY does not activate complement, so it is safer than other antibodies. Compared with IgG, IgY has more stable physical chemical properties and heat resistance: it is stable at 60-70℃ for 5 years and stable at 4℃ for 6 months at room temperature (33,34), It is easy to collect (35) and high in content. Each egg contains 1mg of specific antibody, which is equivalent to the content of the whole blood of a rabbit (36). Further, it is Acid-and enzyme-resistant and can be administered orally (37,38), which is expected to play an important role in preventing and treating SARS-CoV-2 infection.

## Methods

### 1. Preparation of antigen

An enterokinase site sequence (DDDDK) was added to the N-terminal of RBD peptide, and the corresponding nucleic acid sequence was synthesized with reference to prokaryotic expression preference. The pCMV-DsbC-RBD fusion

expression vector was constructed, and the DsbC-RBD fusion protein was obtained by eukaryotic expression and affinity purification. The fusion protein was digested with enterokinase and purified by secondary affinity to obtain a highpurity SARS-CoV-2 S protein RBD peptide.( Provided by Sina Biological Company) See Fig.2.

## 2. Immunity of hens

SPF 14-week-old white single-crowned Leghorn hens (Provided by SPF Experimental Animal Center of Guangdong Xinxing Dahua Agricultural Poultry Eggs Co., Ltd.) were reared in the environment of 20-25℃, 60-90% humidity, natural light and 100-level air purification. The food and water were disinfected. After 4 weeks of adaptive rearing and 2 weeks after laying eggs, the hens began to be immunized, and the RBD protein antigen was mixed with the same amount of Freund's incomplete adjuvant and fully emulsified for later use (the amount of antigen is preferably 400μg / a chicken / time).Intramuscular injection of 0.25ml in each part was given to the armpit of bilateral chicken wings and the left and right sides of abdomen, and immunization was carried out once every 7 days for 4 times.

## 3. Extraction and separation of IgY

Separated egg yolk with egg separator, diluted egg yolk with 9 times of water, stirred well, adjusted pH of egg liquid to 5.0-5.2 with 0.1 mol/L HCl, and then stood at 4℃ overnight. Freeze-centrifuge at 4000rpm for 40min, added

$(NH4)_2SO_4$ to the supernatant to make its final saturation 45%, and stood at 4℃ for 3h; freeze-centrifuge at 4000rpm for 10min, centrifuge at 4000rpm for 10min, discarded the supernatant, added water to dissolve the protein precipitate, added $Na_2SO_4$ (the final mass fraction is 13%) to fully dissolve it, and then stood at 4℃ for 3h; freeze-centrifuge at 4000rpm for 10min, discarded the supernatant, and added PBS for dissolution. Dialyzed the solution in a dialysis bag for 4-5 hours and stored it at -20℃.

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## 4. SDS-PAGE

Prepared 10% SDS-PAGE separation gel according to the formula of the conventional process, and completed it by loading, electrophoresis, dyeing, decolorization, gel imaging and so on(See Fig.3.).

## 5. Western blot

SARS-CoV-2 S-RBD (provided by Sina Biological Company) was separated by polyacrylamide amine gel (10% separation gel+5% concentration gel) under the electrophoresis condition of 50 mA for 60 min. The protein on the gel was transferred to the NC membrane by wet transfer method. The NC membrane was immersed in the 5% sealing solution, then placed on a shaker and sealed for 1.5h at room temperature. After sealing, TBST was used to wash the membrane for 3 times. SARS-CoV-2 -IgY (dilution ratio: 1:10000) was incubated overnight by inversion method. TBST was used to wash the membrane for 5 times, 10min each time, The membrane was immersed and sealed in sealing solution for 30min at room temperature.The second antibody was prepared by diluting HRP-labeled goat anti-chicken(Provided by ZSGB-Bio) with a ratio of 1:10000, and incubated for 1.5 h at room temperature, TBST washed the membrane for 5 times, The mixed immunoblotting chemiluminescence solution was dripped on NC membrane, incubated at room temperature for 2-3 minutes, then placed in a cassette, and the X-ray film was developed (See Fig.4).

## 6. Cells and viruses

The 293T, Vero E6 were obtained from ATCC and maintained in DMEM (Gibco) supplemented with 10% foetal bovine serum. The pseudotyped VSV-ΔG viruses expressingluciferase reporter were provided by Prof. Ningshao Xia, Xiamen University.The SARS-CoV-2 live virus (strain IVCAS 6.7512) was

provided by the National Virus Resource, Wuhan Institute of Virology, Chinese Academy of Sciences.

## 7. Pseudotype virus production

To producepseudotyped VSV-ΔG-Luc bearing SARS-CoV-2 spike protein (pSARS-CoV-2), Vero E6 cells were seeded in 10 cm dish and transfected simultaneously with 15 μg SARS CoV-2-S-Δ18 plasmid by Lipofectamine 3000 (Thermo). Forty-eight hours posttransfection, 150 μl pseudotyped VSV-ΔG bearing VSV-G protein were used to infect Vero E6 cells. Cell supernatants were collected after another 24 hours clearing from cell debris by centrifugation at 3000rpm for 6 minutes, aliquoted and stored at − 80℃.

## 8. SARS-CoV-2 entry assay based on pseudotyped virus

Vero E6 cells were seeded in 48-well plates and added 10ul volumes of pseudotyped VSV-ΔG-Luc bearing SARS-CoV-2 spike protein virus stocks with S-IgY or the control. At 24 h post-pseudotype-infection, the luciferase activities were measured with the Luciferase Assay System (Promega E4550).

## 9. qRT-PCR

One hundred microliter cell culture supernatant was harvested for viral RNA extraction using the MiniBEST Viral RNA/DNA Extraction Kit (Takara, Cat no. 9766) according to the manufacturer's instructions. RNA was eluted in 30 μL RNase-free water. Reverse transcription was performed with a PrimeScript RT

Reagent Kit with gDNA Eraser (Takara, Cat no. RR047A) and qRT-PCR was performed on StepOne Plus Real-time PCR system (Applied Biosystem) with TB Green Premix Ex Taq II (Takara Cat no.RR820A). Briefly, 3 μL total RNA was first digested with gDNA eraser to remove contaminated DNA and then the first-strand cDNA was synthesized in 20 μL reaction and 2 μL cDNA was used as template for quantitative PCR. Receptor binding domain (RBD) of spike gene was amplified by PCR from the cDNA template with primers: RBD-F: 5'-GCTCCATGGCCTAATATTACAAACTTGTGCC3'; RBD-R: 5'-TGCTCTAGACTCAAGTGTCTGTGGATCAC-3', cloned into pCMV-Flag vector (Invitrogen) and used as the plasmid standard after its identity was confirmed by sequencing. A standard curve was generated by determination of copy numbers from serial dilutions ($10^3$-$10^9$ copies) of the plasmid. The primers used for quantitative PCR were RBD-qF1: 5'-CAATGGTTTAACAGGCACAGG-3' and RBD-qR1:5'-CTCAAGTGTCTGTGGATCACG-3. 1 PCR amplification was performed as follows: 95 ℃ for 5 min followed by 40 cycles consisting of 95℃ for 15 s, 54 ℃for 15 s, 72℃ for 30 s(See Fig.5.).

## 10. Immunofluorescence

To detect viral protein expression in Vero E6 cells , cells were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. Then the cells were blocked with 5% bovine serum albumin (BSA) at room temperature for 2 h. The cells were further incubated with the primary antibody (a monoclonal antibody

against viral S protein) for 2 h, followed by incubation with the secondary antibody (Alexa 488-labeled goat anti-mouse[1:500; Abcam]). The nuclei were stained with DAPI dye (Beyotime, China). The images were taken by fluorescence microscopy (See Fig.6.).

## 11. SARS-CoV-2 /VeroE6 cell experiment.

25ul of SARS-CoV-2 (100PFU) was incubated with an equal volume of Pre-IgY,S-IgY and Remdesivir in different concentration gradients for 10 minutes, then added to $1\times10^4$ vero cells, and cultured in an incubator at 37℃ for 4 h, and then the cell culture solution was changed to complete medium. After 48 hours, cells were collected and total RNA was extracted. The whole procedure was performed in a biosafety level (BSL-3) laboratory.After reverse transcription, the copy number of SARS-CoV-2 genome in each sample was detected by qRT-PCR(See Fig.7.).

## Author Contributions

Jingchen Wei and Yunfei Lucontributed equally to this work.

## Conflicts of Interest

All authors declare no conflict of interest.

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## Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 software. Data are expressed as mean ± SD.T-test was used for two-group comparisons. The $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ were considered significant. Unless otherwise noted, error bars are indicated as mean values with standard deviation of at least three experiments.

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## Figure legends

## Fig. 1.

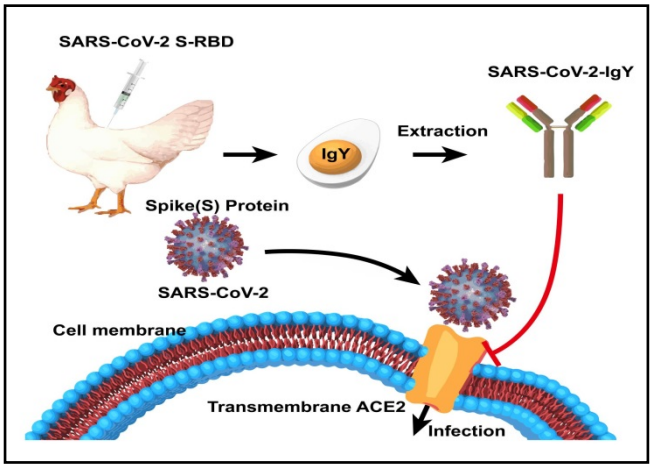


**Fig. 1.Graphical Abstract**

The figure briefly illustrates that the preparation and extraction of S-IgY and its anti-S-CoV-2 mechanism is to inhibit the entry and replication of SARS-CoV-2 by targeting the ACE2.

## Fig.2.

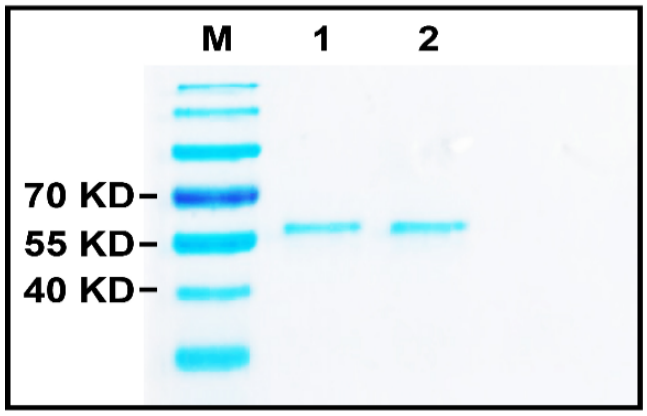


**Fig.2. Gel electrophoresis of SARS-CoV-2-S-RBD**

Fusion protein of SARS-CoV-2 S-RBD was between 70kD and 55kD, in according with the predicted molecular weight 66.2KD was made by the pCMV-DsbC-RBD fusion expression vector (provided by Sina Biological Company) and band one and two showed the purity of the protein was very high.

**Fig.3.**

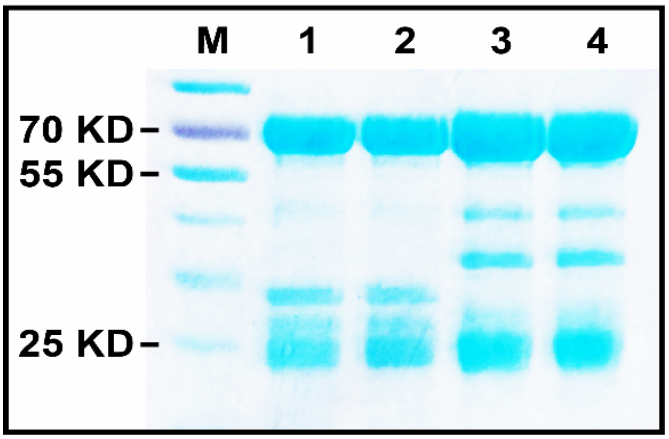


**Fig.3. SDS-PAGE gel detection of S-IgY**

SDS-PAGE gel detection of S-IgY was done which showed that the heavy chain band was at 70kD and the light chain band was at 25kD, and the total IgY purity was about 85%.

**Fig.4.**

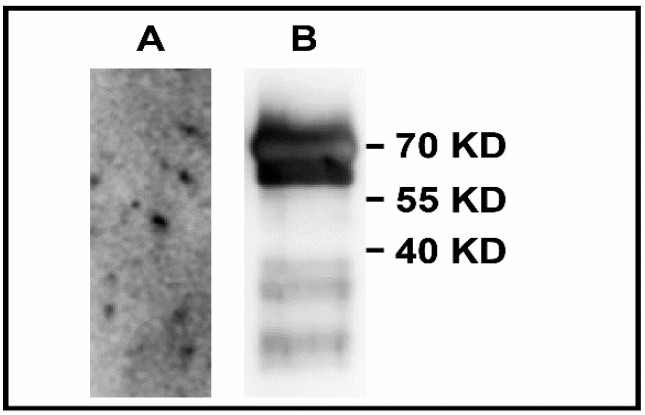


**Fig.4. Detection of SARS-CoV-2-IgY specificity by Western blot**

**A:** Pre-Immu-IgY **B:** SARS-CoV-2–IgY(66.2KD)

The WB results showed that SARS-CoV-2 had a good effect on S-RBD antigen The antibody band was clearly located between 55 KD and 70KD, which was consistent with the electrophoresis position of S-RBD antigen(66.2KD). In this experiment, the dilution of primary antibody (S-IgY) was 1:10000, and the dilution of secondary antibody (HRP-labeled goat anti-chicken) was 1: 10000.

**Fig.5.**

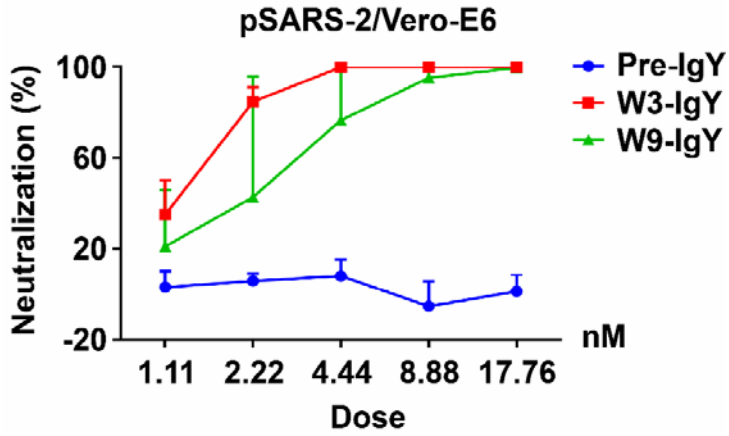


**Fig.5. pSARS-CoV-2/VeroE6 ( qRT-PCR)**

The resultsof viral load in the cells detected by qRT-PCR demonstrated thatS-IgY can efficiently blocking the cell entry of virus, The $EC_{50}$ of W3-IgY is 1.35 ± 0.15 nM and $EC_{50}$ of W9-IgYis 2.76±1.54 nM, and the Pre-IgY has no inhibitory effect on virus.

**Fig.6.**

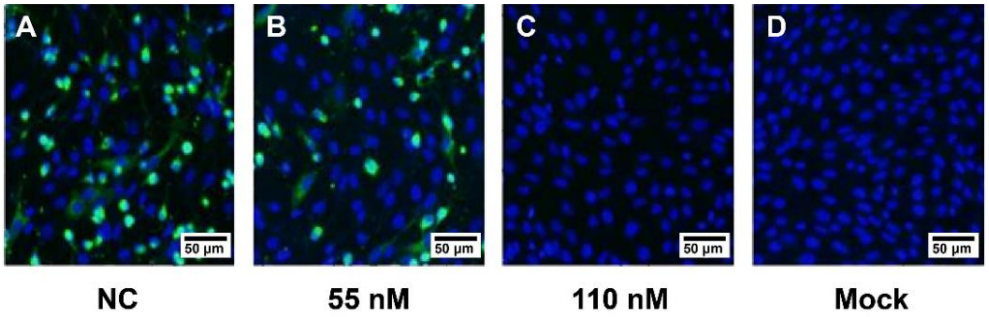


**Fig.6.Immunofluorescence microscopy**

Vero E6 cells were incubated with the S-IgY for 2 h, followed by incubation with the secondary antibody (Alexa 488-labeled goat anti-mouse[1:500; Abcam]). The nuclei were stained with DAPI dye (Beyotime, China). The images were taken by fluorescence microscopy, Results were as follow:

（A） NC：pre-immunized IgY: Full-field virus protein

（B） SARS-CoV-2-IgY55nM：Viral protein decreased significantly in visual field，About 50% of the viruses were suppressed;

（C）SARS-CoV-2-IgY110nM： Virus protein disappeared completely in visual field,The virus was completely suppressed;

（D）Mock Cells without virus addition.

It means that S-IgY can effectively inhibit the SARS-CoV-2 from entering cells and has a dose-dependent relationship.

**Fig.7.**

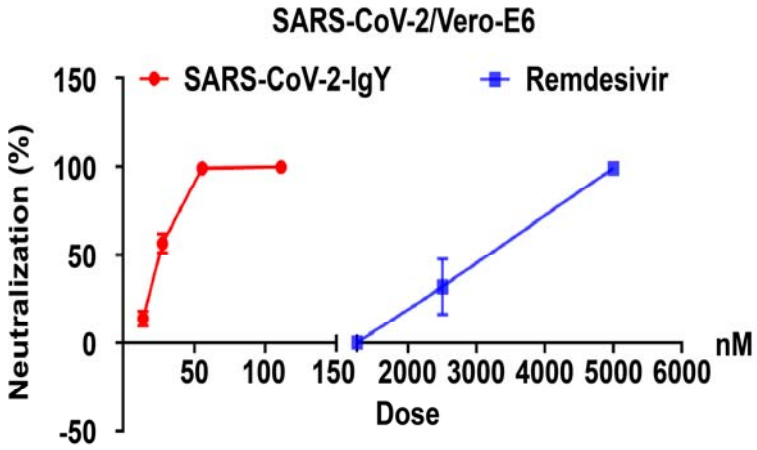


**Fig.7. SARS-CoV-2/Vero-E6 ( qRT-PCR)**

25ul of SARS-CoV-2 (100PFU) was incubated with an equal volume of Pre-IgY, S-IgY and Remdesivir in different concentration gradients for 10 minutes, then added to $1\times10^4$ vero cells, and cultured in an incubator at 37℃ for 4 hours, and then the cell culture solution was changed to complete medium. After 48 hours, cells were collected and total RNA was extracted, the copy number of SARS-CoV-2 genome in each sample was detected by qRT-PCR, $EC_{50}$ of S-IgY is 27.78 ±1.54nM, and $EC_{50}$ of Remdesivir is 3259±159.62 nM,S-2-IgY vs Remdesivir ($P<0.001$). In vitro, the anti-SARS-CoV-2 effect of IgY is 106 times stronger than that of Remdesivir.