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**COVID-19 Ag Test (2019-nCoV) New Coronavirus  
Antigen Test (Colloidal Gold)  
Main Raw Materials Research**

<b>Product Name:</b>	COVID-19 Antigen Test Kit (Colloidal Gold)
<b>Applicant</b>	SINGUWAY BIOTECH INC.
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## 1. Main raw materials used in production processes

The raw materials of this product are divided into main biological raw materials, main biological auxiliary materials, chemical raw materials, auxiliary materials, etc. Since this product is mainly prepared with reference to the colloidal method, the raw material indicators of the colloidal gold related industries may be quoted in this reagent.

## 2. Research on Selection, Preparation and Quality Standards of Main Raw Materials

### 2.1 Main biological raw materials

#### 2.1.1 Reaction Principle

Firstly, a certain amount of a known specific antibody (monoclonal antibody or polyclonal antibody) is coated on the membrane as a detection zone, and a secondary antibody that can bind to the gold label is used as a quality control zone. The gold-labeled conjugate of another monoclonal antibody paired with the coated antibody is adsorbed on the gold-labeled pad and dried. One end of the dry gold label pad is connected to the membrane, and one end is connected to the sample pad. The other of the membrane Stick an absorbent pad on the side. During testing, a certain amount of liquid sample is added to the sample pad, and the sample moves in the direction from the sample pad to the absorbent pad by means of capillary action. It first passes through a dry gold label pad to reconstitute the gold label conjugate. If there is an antigen to be tested in the specimen, an antigen-antibody reaction will occur to form complex A (gold particles-antibody-antigen); the sample continues to move to the detection zone. , The antigen-antibody reaction occurs again with the coating antibody to form complex B (gold particle-antibody-antigen-coating antibody), which will accumulate in the detection zone and finally reach the naked eye level, if there is no sample to be tested Antigen, the red band visible to the naked eye cannot be formed. The free gold-labeled conjugate or complex A crosses the detection zone to the quality control zone, reacts with the secondary antibody, and forms complex C (gold particle-antibody-secondary antibody), aggregates and produces a red band visible to the naked eye. Regardless of whether the sample contains the substance to be tested, the quality control band will show a red band.

#### 2.1.2 Main raw materials

It can be seen from the reaction principle that the main biological raw materials include gold standard antibody, coated antibody and second antibody. Besides, the excipient with great influence on quality is NC film.

Name of component		Main raw materials	Source
Test card	Chip card holder	Plastic casings	Baoli mold plastic products.Inc(Zhuhai,China)
	Scribing	×PBS 10	Self-produced

Name of component		Main raw materials	Source
Lysis Solution	solution	Propanol	Shanghai Aladdin biological technology co., LTD ((Shanghai, China))
		Sodium thiomersal	Shanghai Sagon Biotech.Ltd (Shanghai, China)
		FPZ0548 of antibody coated	Fapon Biotech Inc( Dongguan, China)
		Sucrose	Shanghai Sagon Biotech.Ltd (Shanghai, China)
		BSA	Nanjing Dulai biotech .Ltd(Nanjing,China)
	Quality control solution	10×PBS	Self-produced
		Propanol	Shanghai Aladdin biological technology co., LTD ((Shanghai, China))
		Sodium thiomersal	Shanghai Sagon Biotech.Ltd (Shanghai, China)
		BSA	Nanjing Dulai biotech .Ltd(Nanjing,China)
		Sucrose	Shanghai Sagon Biotech.Ltd (Shanghai, China)
	Gold Standard Antibody	Protein A	Shanghai Sagon Biotech.Ltd (Shanghai, China)
		Colloidal gold	Self-produced
		Gold standard antibody dilution	Self-produced
	Seal	FPZ0546 of labeled antibodies	Fapon Biotech Inc( Dongguan, China)
		Aluminum foil bag	Hebei Yuhua Packaging Inc(Shijiazhaung, China)
Annex	Desiccant (silica gel)	Dongguan Purification( Dongguan, China)	
	Water absorbent paper	Dongguan Jinlili Biotechnology Co., Ltd.(Dongguan, China)	
	NC membrane	Pall Corporation (New York, USA)	
	Sample pad	Dongguan Jinlili Biotechnology Co., Ltd..(Dongguan, China)	
Lysis Solution	RIRA lysate	Self-produced	

### 2.1.3 Selection of antibody pairs

#### 2.1.3.1 Test programme

We screened three groups of antibodies from feipeng organisms; the FPZ0573/FPZ0546 antibody pairs (antibody to 1), FPZ0548/FPZ0546 antibody (antibody to 2), and FPZ0550/FPZ0554 antibody pairs (antibody to 3) were tested. The antigen epitopes of the new coronavirus and the corresponding negative specimens were selected for testing.

#### 2.1.3.2 Criteria

The antibody pair with the highest titer ratio was selected as the antibody pair in this experiment.

#### 2.1.3.3 Experimental results

Sample	Antibody	1.0 ug/mL	25 ng/mL	ng/mL 2	0.5 ng/mL	0.1 ng/mL	1.0 ug/mL	25 ng/mL
Weak positive	1	+	+	+	+	+	-	-
	2	+	+	+	+	+	+	-
	3	+	+	+	+	+	-	-
Positive	1	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+

Sample	Antibody	1.0 ug/mL	25 ng/mL	ng/mL 2	0.5 ng/mL	0.1 ng/mL	1.0 ug/mL	25 ng/mL
	3	+	+	+	+	+	+	+
Negative	1	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-

2.1.3.4 results analysis

The results showed that antibody 2 had higher activity on the combination. Therefore, antibody pair 2 was selected as antibody combination for the detection of this reagent.

Verification of 2.1.4 antibody pairs

- (1) Verification method: the antibody pair is directly combined with other excipients to verify the antibody response.
- (2) Validation results: The results showed that antibody pairs could react.



**2.1.4 Selection of Quality Control Protein**

2.1.4.1 Pilot programme

The mouse anti-human IgG and protein A were diluted and marked with gold marker FPZ0546 as marker protein to observe the immunofiltration reaction.

2.1.4.2 Experimental results

Dilution factor	1: 1	1: 2	1: 4	1: 8	1: 16	1: 32
protein A	+	+	+	+	+	+
Anti-human IgG	+	+	+	+	+	+

According to the results, both of them have high titer ratio, considering the generality of Protein A, they are selected as the quality control protein of this reagent.

2.1.4.3 Protein A quality standards

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Performance test: after protein A marking, use FPZ0546 as marking pad, quality control line should be able to show color.

#### 2.1.4.4 Verification of quality control products

- (1) Verification scheme: FPZ0546 as a marking pad, protein A line, should appear color reaction.
- (2) Verification results:



- (3) Conclusion

The Protein A produced by Sangon can be used as the quality control protein of this reagent.

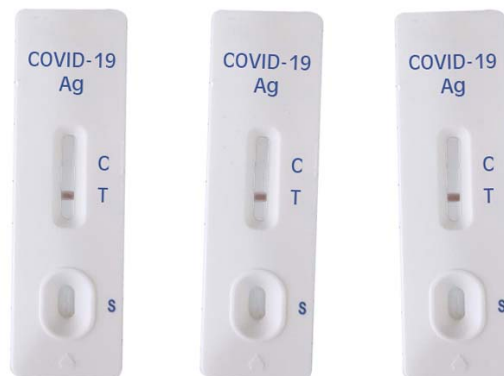
#### 2.1.5 Selection of lysate

##### 2.1.5.1 Test programme

Select several common lysates to react as lysates of this reagent, including PBS and RIPA lysates. RIPA lysate is used as the lysate of this reagent. Take the positive inactivated specimen as the sample to observe whether the lysate will cause the reaction.

##### 2.1.5.2 Test results

The results are as follows:



As a result, RIPA buffer can be used as the lysate of this reagent.

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## **2.2 Selection of main auxiliary materials**

### **2.2.1 Selection and quality standard of NC film**

This kit selects the 170membrane of Pall company as the NC membrane of the reagent.

### **2.2.2 Verification scheme**

- A. Appearance observation: Soft and meticulous.
- B. Functional verification : In the performance test, C, T should be able to develop color after the three antigens are crossed and tested with reference substance.

### **2.2.3 Verification results**

The appearance meets the requirements, the water absorption speed is 10mm /min, and the color development is normal.



### **2.2.4 Verification conclusion**

The NC170membrane of Pall can be used as the membrane of this reagent.