

## Justification

Regarding the self testing product Coronavirus (2019-nCoV) -Antigentest- produced by Beijing Hotgen Biotech Co., Ltd., the following justifications are especially made:

1. The Coronavirus (2019-nCoV) -Antigentest- by our company detects N protein of SARS-CoV-2.

2. The current mutation position of the Novel Coronavirus mutant strain (B.1.1.7 strain) are D3L and S235F, (B.1.618 strain) are E484K and D614G.

Of the two antibodies we used, one of them has a recognition site of 75-119aa; the other has a recognition site of 45-181aa. The epitope recognized by our antibody does not contain mutated epitopes. While the mutation of the virus found in England (B.1.1.7 strain), South Africa (501Y.V2 strain), Brazil (P.1 strain) and India (B.1.618 strain) are mainly on S protein. So our product is still effective to detect the antigen of novel coronavirus and the mutation will not affect the detection result of our product.

The following is the SARS-COV-2 N Protein Identification Report.

## Annex 1

### SARS-CoV-2 N Protein Identification Report

**Test name:** SARS-CoV-2 N monoclonal antibody characterization

**Test method:** ELISA test

**Test materials:** Full-length antigens and different N protein truncated antigens, synthetic peptides

**The antigens are:** pet32-1-420aa, pet32-45-181aa, pet32-188-258aa, Pet32-259-419aa;

**The synthetic peptides are:** 2-50aa, 45-89aa, 75-119aa, 105-149aa, 135-181aa

**Test steps:**

1. Different antigens are coated with 300ng/ml, synthetic peptides are coated with 5ug/ml (coating buffer 20mM CB9.6), 37°C for 2 hours, 4°C overnight.
2. Wash the plates once, seal with commercial blocking buffer, 37°C for 2h.
3. Dilute the two antibodies to 10µg/ml respectively, use different antigens and synthetic peptide plates for detection, the reaction mode is 30-30-10min.

**Test results 1:**

Antigen Antibody	pet32-1-420aa	pet32-45-181aa	pet32-188-258aa	pet32-259-419aa
Ab-1	+	+	-	-
Ab-2	+	+	-	-

**Test results 2:**

Antigen Antibody	2-50aa	45-89aa	75-119aa	105-149aa	135-181aa
Ab-1	-	-	+	-	-
Ab-2	-	-	-	-	-

**Conclusion:**

1. Ab-1 recognized epitope is 75-119aa; Ab-2 recognized site is 45-181aa.
2. The current mutation position of the Novel Coronavirus mutant strain (B.1.1.7 strain) is (D3L, S235F), and the epitope recognized by our antibody does not contain these two amino acids.

## Annex 2

### SARS-CoV-2 N Protein Identification Report

**Test name:** Identification of the binding activity of SARS-COV-2 N raw material to N protein mutants

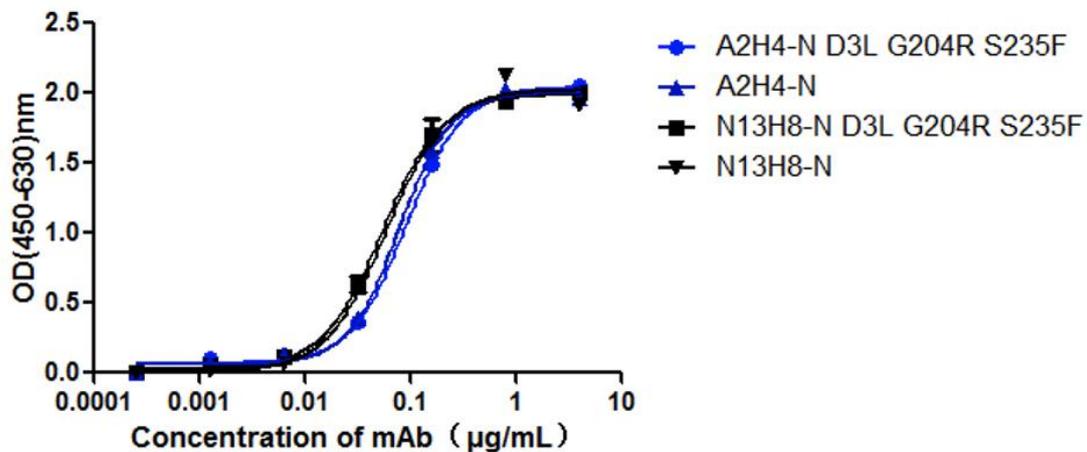
**Main materials:** coated antibody and labeled antibody; Recombinant N protein; Recombinant N protein mutant (D3L G204R S235F); HRP-Goat Anti-Mouse IgG

**The main steps:**

1. Different antigens are coated with 4 $\mu$ g/mL (coating buffer 20mM PB pH7.0), 37 $^{\circ}$ C for 1h. 200 $\mu$ L of blocking buffer per well was blocked at 37 $^{\circ}$ C for 2h.
2. A2H4 and N13H8 antibodies were diluted 5-fold from 4 $\mu$ g/ml with 6 concentration gradients, combined at 37 $^{\circ}$ C for 30 minutes, and washed the plates.
3. HRP-Goat Anti-Mouse IgG binding, 37 $^{\circ}$ C for 30 minutes. Wash plates and develop coloration.

**Test results:**

Antibody and N protein mutant binding activity



**Conclusion:** The binding activity of A2H4/N13H8 antibody raw material and N protein mutant D3L G204R S235F is not different from that of N protein parent.

