

# HyTest SARS-CoV-2 antibodies and detection of variants

## Anti-nucleoprotein antibodies (Cat.# 3CV4)

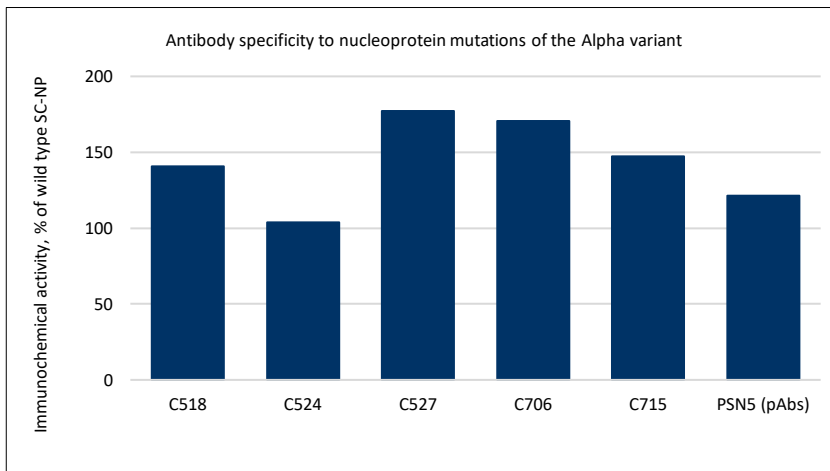
All HyTest SARS-CoV-2 nucleoprotein (NP) antibodies (Cat.# 3CV4) including MAb C706, C524, C518, C715, and C527 bind to the N-terminal part of the nucleoprotein, N47-A173. Several mutations found in circulating SARS-CoV-2 variants are located outside of this region, however, we aim to test all relevant and concerning mutations regardless of their location. Prevalence of nucleoprotein mutations changes over time, for up-to-date information see e.g. <https://outbreak.info/compare-lineages>.

We screen for the variants ourselves using recombinant antigens, however, we also have received feedback from our customers that have tested selected antibody pairs with live virus variant strains. We highly appreciate the feedback from our customers as it gives valuable information on the performance of some of the pairs with live virus samples.

Below we have compiled information regarding different variants and mutations. We continue to update this information regularly. **To minimize the likelihood that a COVID-19 antigen test will fail to recognize emerging variants, we recommend using more than two antibodies in the assay (see pages 5 and 6.)**

Mutations	Variant	Hytest antibodies
D3L, R203K, G204R, S235F	<b>Alpha (B.1.1.7)</b>	<ul style="list-style-type: none"><li>• Recognized by all Hytest MAbs (Cat. # 3CV4) and pAb (Cat. # PSN5). See Figure 1.</li><li>• Performance of selected pairs confirmed with patient samples/live virus variants:<ul style="list-style-type: none"><li>○ C524-C706, C518-C706 (see Table 1)</li><li>○ C715-C706</li></ul></li></ul>
T205I	<b>Beta (B.1.351); Epsilon (B.1.427) and B.1.429)</b>	<ul style="list-style-type: none"><li>• See Table 1 and Figure 3</li></ul>
P80R, R203K, G204R	<b>Gamma (P.1)</b>	<ul style="list-style-type: none"><li>• Two of three mutations are same as in the Alpha variant</li><li>• P80R mutation: see Figure 3</li><li>• Performance confirmed with live virus variant for C715-C706</li></ul>
D63G, R203M, D377Y	<b>Delta (B.1.617.2)</b>	<ul style="list-style-type: none"><li>• D63G see Figure 3</li><li>• Two other mutations located outside the epitope of our key clones</li></ul>
R203M, D377Y	<b>Kappa (B.1.617.1)</b>	<ul style="list-style-type: none"><li>• Mutations located outside the epitope of our key clones</li><li>• It is expected that all our antibodies recognize also the kappa variant</li></ul>
P13L, D31-33, R203K, G204R	<b>Omicron (B.1.1.529)</b>	<ul style="list-style-type: none"><li>• Mutations located outside the epitope of our key clones</li><li>• It is expected that all our antibodies recognize also the omicron variant. See Figure 2.</li></ul>

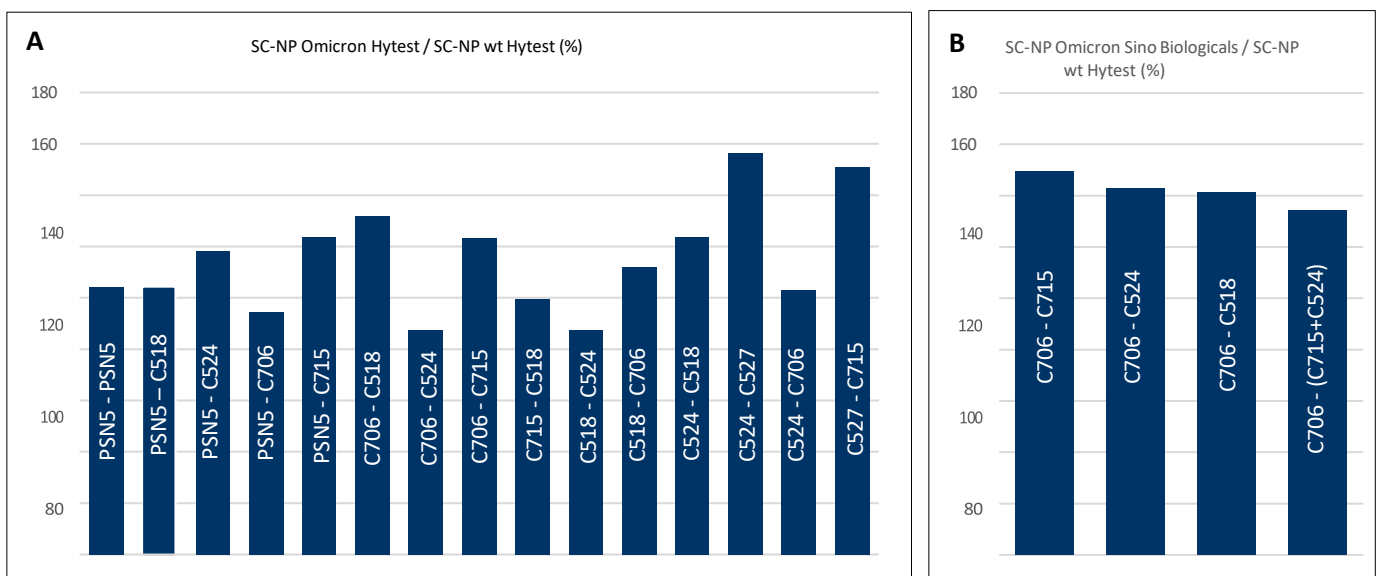
P13L, del31/33, R203K, G204R, S413R	<b>Eris (EG.5)</b>	<ul style="list-style-type: none"> <li>Hytest SARS-CoV-2 NP antibodies (Cat. # 3CV4: MAbs C706, C524, C518, C715, and C527) should recognize Pirola and Eris variants.</li> </ul>
P13L, del31/33, R203K, G204R, S413R	<b>Pirola (BA.2.86);</b>	
P13L, del31/33, P151S, R203K, G204R, S413R	<b>Omicron (BA.4)</b>	<ul style="list-style-type: none"> <li>All our key antibodies recognize also the omicron BA.4 variant</li> <li>Hytest compared SARS-CoV-2 Omicron BA.4 NP with wild-type (original) NP and found no differences in recognition by MAb C706, C524, C518, C715, and C527</li> <li>See Figure 4</li> </ul>
P13L, del31/33, R203K, G204R, Q229K, S413R	<b>FLIRT group (KP.3, KP.3.3, KP.2, and KP.1.1)</b>	<ul style="list-style-type: none"> <li>No additional mutations in N47-A173 part, thus, Hytest SARS-CoV-2 NP antibodies (Cat.# 3CV4: MAb C706, C524, C518, C715, and C527) should recognize FLIRT group variants KP.3, KP.3.3, KP.2, and KP.1.1, as well as similar new variants LB.1, KS.1.1 and XEC variant.</li> </ul>
P13L, del31/33, R203K, G204R, Q229K, S413R	<b>LB.1; KS.1.1; XEC (combination of KS.1.1 and KP.3.3)</b>	
Single mutations	<b>Several variants</b>	<ul style="list-style-type: none"> <li>See Figure 3</li> </ul>



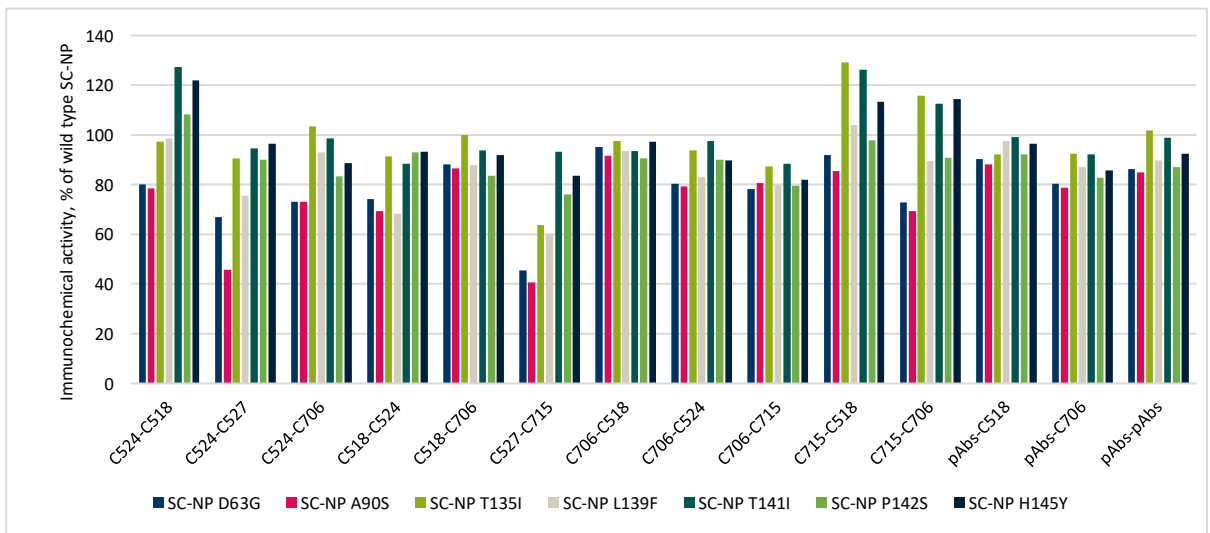
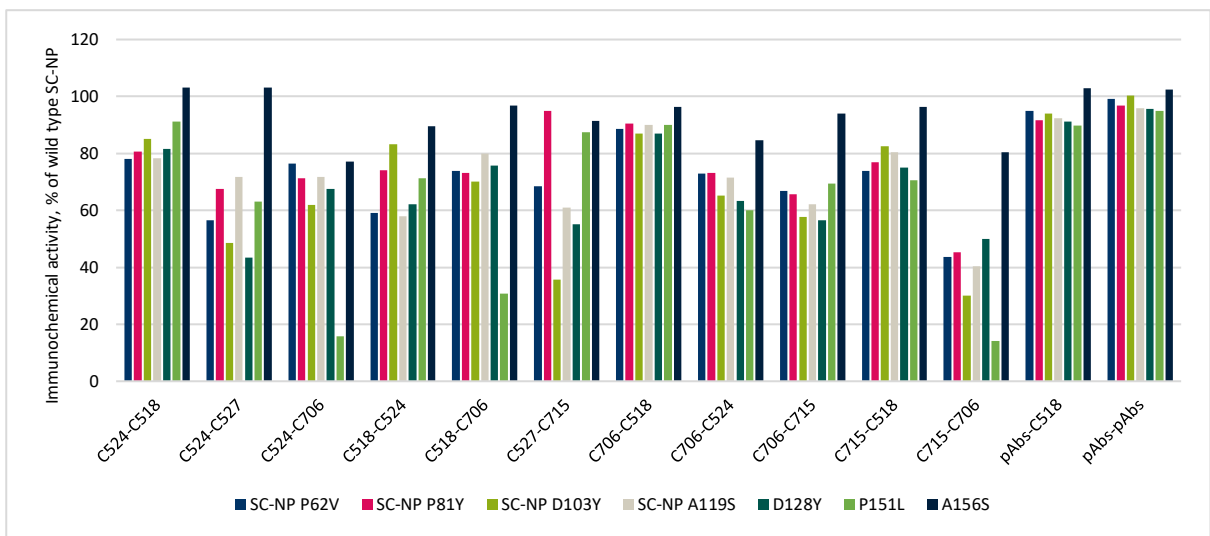
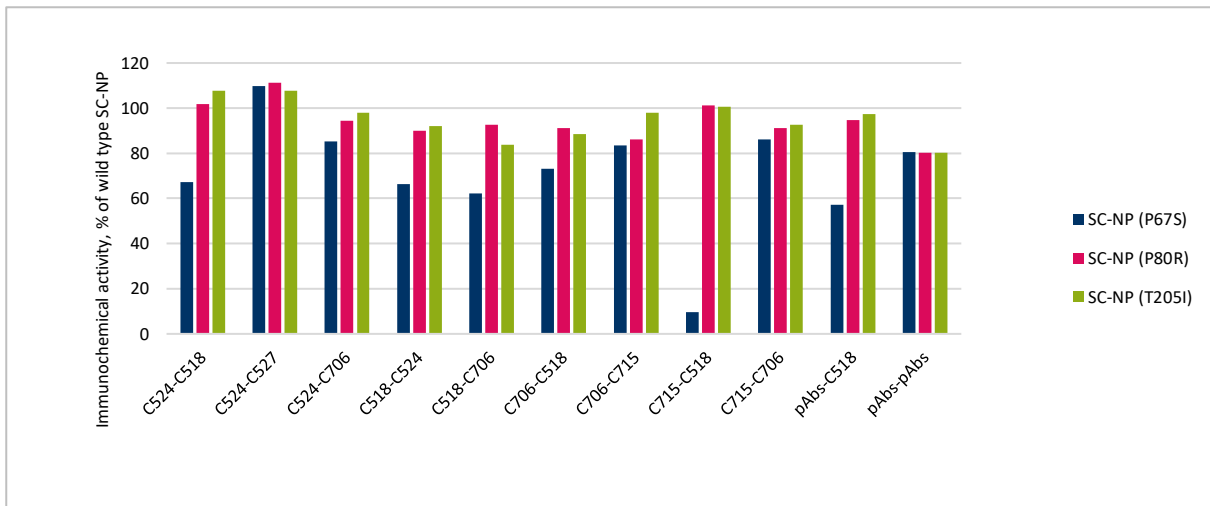
**Figure 1. Detection of the recombinant Alpha variant NP in indirect ELISA.** Signal from recombinant wild type NP (Cat. # 8COV3) was considered as 100%. Recombinant antigen used was 40588-V07E7 from Sino Biological.

**Table 1. Detection of SARS-CoV-2 virus variants tested with two recommended pair combinations on lateral flow.** Swab samples were used as specimen. Note that line intensity depends on virus load that can vary between samples. The results show that these two wt pairs detect all three virus variants in lateral flow. Source: Customer data kindly provided with permission to show.

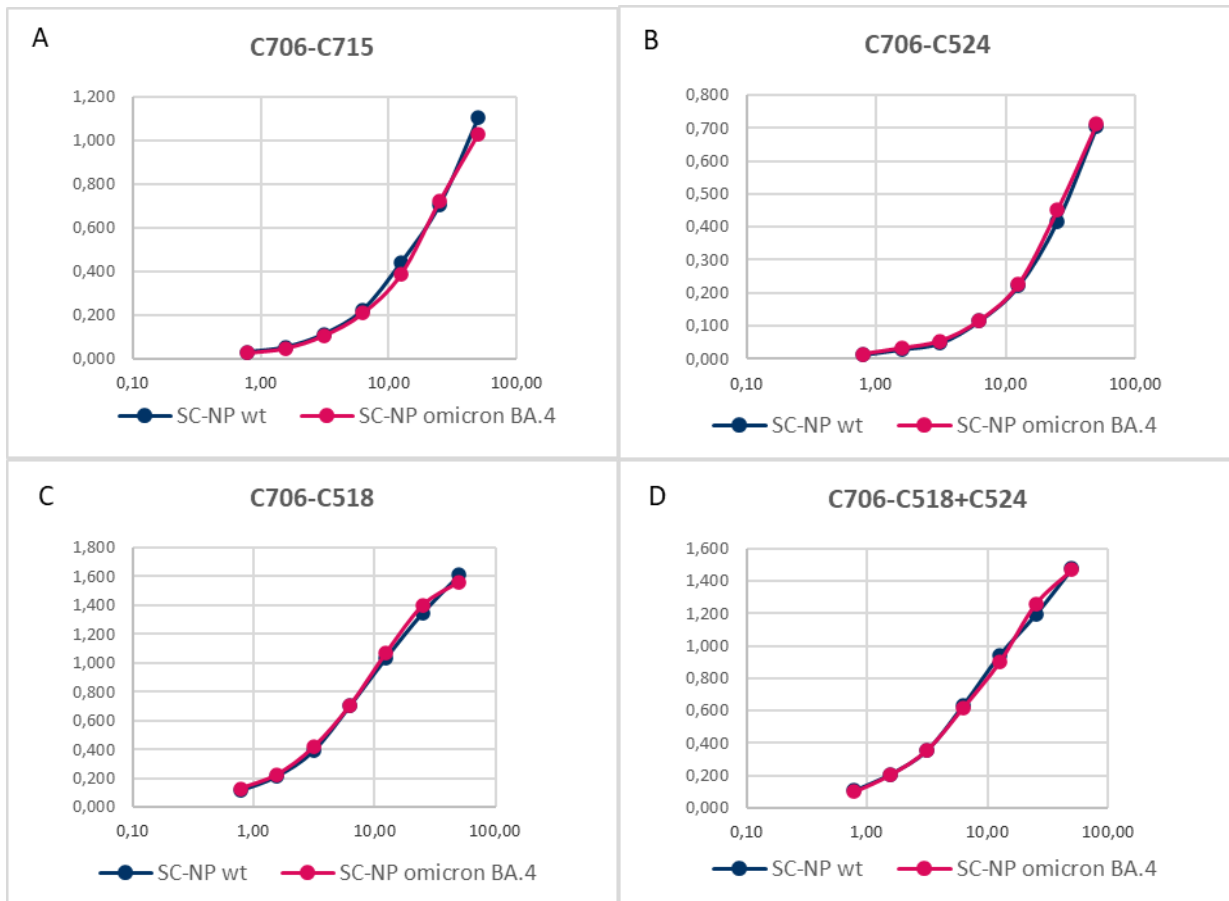
	Variant	MAb pair Line intensity (Capture-Detection);	
		C524-C706	C518-C706
Sample 1	Beta (B.1.351) or Gamma (P.1)	6/10	4/10
Sample 2	Alpha (B.1.1.7)	8/10	7/10
Sample 3	Alpha (B.1.1.7)	8/10	7/10
Sample 4	Beta (B.1.351) or Gamma (P.1)	5/10	4/10



**Figure 2. Detection of the recombinant Omicron variant NP with anti-SC-NP MAbs sandwich antibody pairs.** Signal from recombinant wild type NP (Cat. # 8COV3) was considered as 100%.  
A) Recombinant Omicron antigen used was Hytest inhouse preparation.  
B) Recombinant Omicron antigen used was Sino Biologicals antigen.



**Figure 3. Specificity of anti-SC-NP MAb sandwich antibody pairs to different point mutations.** Signal from recombinant wild type NP (Cat.# 8COV3) was considered as 100%.



**Figure 4. Specificity of anti-SC-NP MAbs sandwich antibody pairs to omicron BA.4 variant.**

Comparison of recombinant wild type NP (Cat.# 8COV3) and SC-NP omicron BA.4 variant using HyTest A) C706-C715, B) C706-524, C) C706-518, and D) C706-C518+C524 in-house assays.

### Pair recommendations

While all 1+1 (capture-detection) pair combinations we have tested are able to detect the key variants currently circulating in the world, we recommend using more than two clones in a COVID-19 Antigen test. This will increase the likelihood of the test to efficiently recognize all future variants too. Also, including anti-SC-NP polyclonals (Cat.# PSN5) as a capture antibody could help.

Sandwich immunoassays			
Detection antibody conjugated with HRP		Detection antibody conjugated with biotin	
Capture	Detection	Capture	Detection
C524	C706	C524	C706

C518	C524	C706	C518
C524	C527	C715	C518
C715	C706	PSN5	C518
C527	C715	PSN5	C527
		C524	C527
		C518	C706
		C524	C518

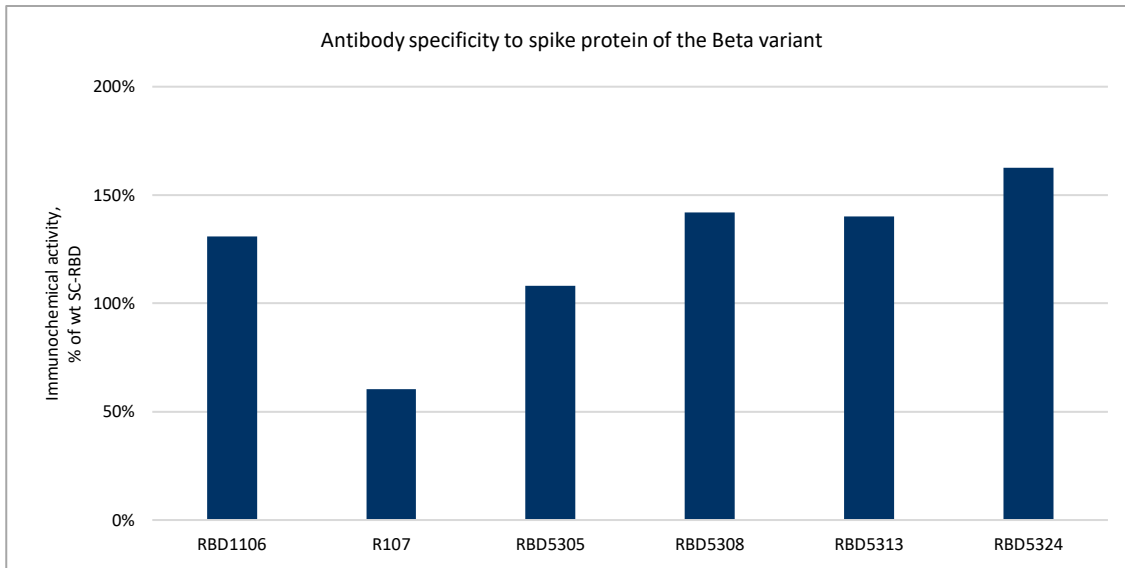
Lateral flow			
+		Advanced	
Capture	Detection	Capture	Detection
C715	C706	C706	C518+C524
C706	C524	C706	C518+C524+C715
C706	C518	C706+C518	C524
PSN5	C518	C706+C518	C524+C715
PSN5	C524	C518+C524	C706
PSN5	C706	C518+C524+C715	C706
C518	C706	C524+C715	C706+C518
C524	C706	C518+C524+C715	C706+C527
C706	C715	C706+PSN5	C518+C524

### Anti-Spike RBD antibodies (Cat. # 3CV2)

The Alpha variant has deletions and mutations within the spike protein, one of which is within the RBD part of the protein (N501Y). We have confirmed that all our anti-RBD clones detect the N501Y variant (recombinant antigen) similarly to the wild type virus.

The Beta variant bears four mutations in its spike protein (K417N, E484K, N501Y, D614G). Our anti-Spike clones recognize recombinant variant spike protein similarly to wild type virus.

The Gamma variant contains three mutations in its spike protein (K417N, E484K, N501Y). Same mutations are found in the South African variant and based on results with the South African variant protein it is expected that our anti-RBD antibodies recognize the Brazilian variant similarly to the wild type.



**Figure 5. Detection of the Beta variant spike (recombinant antigen) with Hytest anti-RBD antibodies.** Signal from recombinant wild type RBD (Cat.# 8COVI) was considered as 100%.