TB Technical Bulletin

NOVACYT GROUP

Variant Testing Update: SNPsig® SARS-CoV-2 EscapePLEX™

The SNPsig SARS-CoV-2 EscapePLEX™ kit is a qPCR test for the detection and differentiation of SARS-CoV-2 variants: Beta, Gamma, Delta, Delta with K417N and Omicron.

It uses two SARS-CoV-2 gene targets (ORF1ab and M gene) to confirm infection, whilst screening for the following clinically significant mutations associated with variants of concern (VoCs):

- E484K
- K417N
- K417T
- P681R

EscapePLEX™ can be used on patient samples from people of all ages at any point during active infection whether symptomatic or asymptomatic to facilitate mass population screening.

Emergence of the Omicron Variant

The Omicron variant (B.1.1.529) was first identified in Botswana in late November 2021. This SARS-CoV-2 variant has more mutations than other variants, including more than 30 mutations in the Spike protein. The Spike protein is the surface protein which interacts with a cell surface receptor to enter the cell and replicate. The emergence of this variant immediately sparked alarm from health experts and governments for its potential to escape the immune system and vaccine.

In the presence of the Omicron variant, EscapePLEX™ will produce a K417N-positive, E484K-negative, K417T-negative, and P681R-negative mutation profile. This mutation profile can be used to distinguish Omicron from other variants of concern (VoCs).

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FAM (E484K)	HEX (K417N)	ROX (K417T)	Cy5 (P681R)	Result	Indicative of:
-	+	-	-	Positive for K417N	Omicron
-	-	-	+	Positive for P681R	Delta
-	+	-	+	Positive for K417N and P681R	Delta with K417N
+	+	-	-	Positive for E484K and K417N	Beta
+	-	+	-	Positive for E484K and K417T	Gamma

Table 1. *Table showing the differential mutation profiles exhibited by Beta, Gamma, Delta, Delta with K417N and Omicron SARS-CoV-2 variants with EscapePLEX™.*

Divergence of the Omicron Variant

On 7 December 2021, the PANGO* committee accepted a proposal to split the variant into two sub-lineages: BA.1 and BA.2. These two sub-lineages differ at several mutation sites. Key amongst these is the Δ69/70 mutation; a deletion at amino acids 69 and 70 in the Spike (S) protein.

Public health agencies have suggested using the Δ69/70 mutation as a first-line screen for Omicron during primary testing. This is because the Δ69/70 mutation is responsible for the S gene target failure (SGTF) phenomenon observed with some SARS-CoV-2 qPCR assays. When using these assays, samples producing a SARS-CoV-2 positive result with SGTF are marked as likely Omicron and prioritised for sequencing.

However, there is a critical difference between the 2 sub-lineages at this mutation site:

- BA.1 contains the Δ69/70 mutation
- BA.2 does not contain the Δ69/70 mutation

The implication of this is that any strategy relying on the SGTF to screen for Omicron may overlook the BA.2 sub-lineage and subsequently under-report Omicron's population distribution. Therefore, the use of SGTF as a primary screening tool may no longer be a safe long-term strategy for controlling the spread of the Omicron variant.

**Phylogenetic Assignment of Named Global Outbreak LINeages.*

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Detecting Omicron with EscapePLEX™

EscapePLEX™ can be used to detect both BA.1 and BA.2 sub-lineages of Omicron. Though it cannot differentiate between the two sub-lineages, it can be used as an effective reflex testing strategy to triage SARS-CoV-2 positive samples for sequencing.

Confidence in the predictive capacity of SNPsig® SARS-CoV-2 EscapePLEX™ was assessed via an in silico evaluation of 5520 SARS-CoV-2 sequences (downloaded from the GISAID EpiCoV database on 13th December 2021). These sequences represented 920 of the most recent submissions to the public databases for each of the six following variants: Alpha, Beta, Delta, Gamma, Mu, and Omicron.

The sequences were analysed to determine whether the "K417N-positive, E484K-negative and P681R-negative" mutation profile correctly identified the Omicron variant. Based on this analysis we determined that the EscapePLEX™ Omicron mutation profile produces an overall percentage agreement (OPA) of 98.9% with the GISAID EpiCoV database.

GISAID EpiCoV Variant Classification	Total Number of Sequences Analysed	Results	
		Number of Sequences containing the EscapePLEX™ Omicron Mutation profile	Number of Sequences negative for the EscapePLEX™ Omicron Mutation profile
Omicron	920	878	42*
Alpha	920	0	920
Beta	920	17	903
Gamma	920	0	920
Delta	920	0	920
Mu	920	0	920

Table 2. *Table showing the numbers of sequences from the GSAID EpiCoV database exhibiting the EscapePLEX™ Omicron mutation profile.*

**Please note: these sequences were classified as Omicron in the public databases but did not harbour all mutations associated with the B.1.1.529 (Omicron) lineage, as reported by the UKHSA Technical Briefing 31.*

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Overall Percentage Agreement Calculation

Overall Percentage Agreement	=	Total number of Omicron sequences correctly identified by the EscapePLEX™ mutation profile	+	Total number of sequences correctly identified as "not Omicron" by the EscapePLEX™ mutation profile

Total Number of Sequences Analysed

=	878	+	4,583	
		5,520		
=		98.9%		

Product Codes

• SNPsig® SARS-CoV-2 EscapePLEX™ CE IVD:	D00152
• SNPsig® SARS-CoV-2 EscapePLEX™ RUO:	R00152

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CE IVD

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