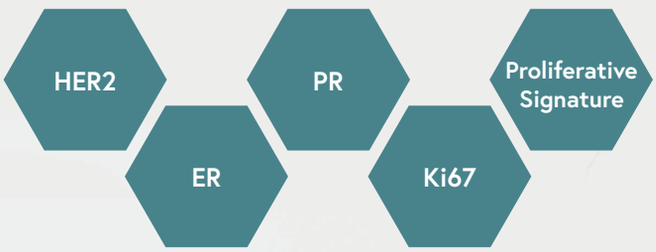
APIS Breast Cancer Subtyping Kit

APIS
Assay Technologies Ltd.

An advanced assay for characterising breast cancer, providing **fast** and **highly accurate results**



Understanding Breast Cancer Tumour Biology

Molecular subtyping is critical for therapy selection

Receptor tyrosine-protein kinase erbB-2 (HER2), oestrogen receptor (ER), progesterone receptor (PR), and marker of proliferation Ki67 expression plays a key role in breast cancer intrinsic subtype evaluation and guides treatment decisions.

The APIS Breast Cancer Subtyping Kit is an RNA-based diagnostic workflow for detecting mRNA expression of standard biomarkers (HER2, ER, PR, Ki67) and a novel proliferative signature from pre-operative core needle biopsy (CNB) or resected formalin-fixed paraffin-embedded (FFPE) breast tumour tissue. The test serves as an alternative method to current standard of care – immunohistochemistry (IHC).

Breast Cancer Surrogate Intrinsic Subtype Classification*

Breast Cancer Subtypes	HER2	ER	PR	Ki67	Recommended Treatment
Luminal A-like	Negative	Positive or Negative	Positive or Negative	Low	Endocrine therapy
Luminal B-like (HER2 negative)	Negative	Positive	Positive or Negative	High	Endocrine therapy ± Chemotherapy
Luminal B-like (HER2 positive)	Positive	Positive or Negative	Positive or Negative	Low or High	Chemotherapy + Anti-HER2 therapy + Endocrine therapy
HER2 enriched (non-luminal)	Positive	Negative	Negative	Low or High	Chemotherapy + Anti-HER2 therapy
Triple negative	Negative	Negative	Negative	Low or High	Chemotherapy

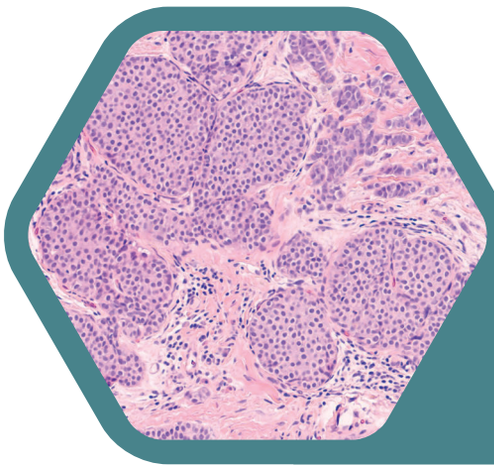
Breast Cancer Diagnostic Needs

	Need	APIS Breast Cancer Subtyping Kit
Patients / Clinicians	Accuracy; Low HER2 stratification	Aids patient management, providing accurate results
Histopathologists	Accuracy; Reduced workload	Supports workload
Molecular oncology lab technicians	Precision and reproducibility	Delivers reliable results to healthcare professionals
Healthcare executives	Resource (time and cost)	Improves service with little added cost

Cardoso F, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2019;30(8):1194-1220

APIS Breast Cancer Subtyping Kit

Delivering **precise results** for standard biomarkers and a novel proliferative signature in **less than 5 hours***



Sample Preparation

- 10µm FFPE tissue section (tumour content ≥ 20%)

Recommended RNA Extraction & Normalisation

- Use of RNeasy® DSP FFPE Tissue Kit
- Fluorometric RNA Quantification

APIS Breast Cancer Subtyping Kit Setup

- Master mix prep and plate setup
- Up to 10 patient samples per plate

RT-qPCR Reaction

- Validated on QuantStudio™ 5 Dx instrument

Analysis and Results Reporting

- Analysis using automated APIS Breast Cancer Subtyping Kit Software with the results available in seconds

* for processing 10 samples

Automated Breast Cancer Subtyping Results Interpretation

Enabling an **understanding** of tumour's molecular profile

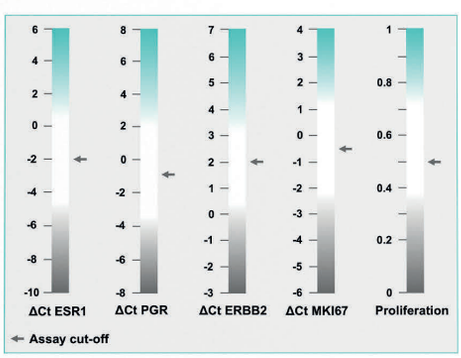
Gene-level nomenclature, HER2 = ERBB2, ER = ESR1, PR = PGR, Ki67 = MKI67

Sample: Example
Run name: Example
Validity: Valid

Marker	Status	dCt
ESR1	Negative	-5.22
PGR	Negative	-0.68
ERBB2	Negative	1
MKI67	High	1.45
Proliferation	High	0.78
Subtype: Triple Negative		

Qualitative (positive/negative) and semi-quantitative (ΔCt) single biomarker status, as well as surrogate subtype

Measure of proliferation (high/low), determined through a novel four-gene signature



Arrow indicates assay ΔCt cut off

Visualise biomarker mRNA expression on a ΔCt scale relative to validated cut-offs

Advantages of the APIS Breast Cancer Subtyping Kit

- Detecting HER2, ER, PR & Ki67 mRNA expression with high precision and reproducibility
- Single HER2 resolution method, without the need for reflex testing
- Novel four-gene signature for measuring proliferation, supporting use of Ki67 alone
- Automated results interpretation with APIS software
- Opportunity to enhance HER2 stratification

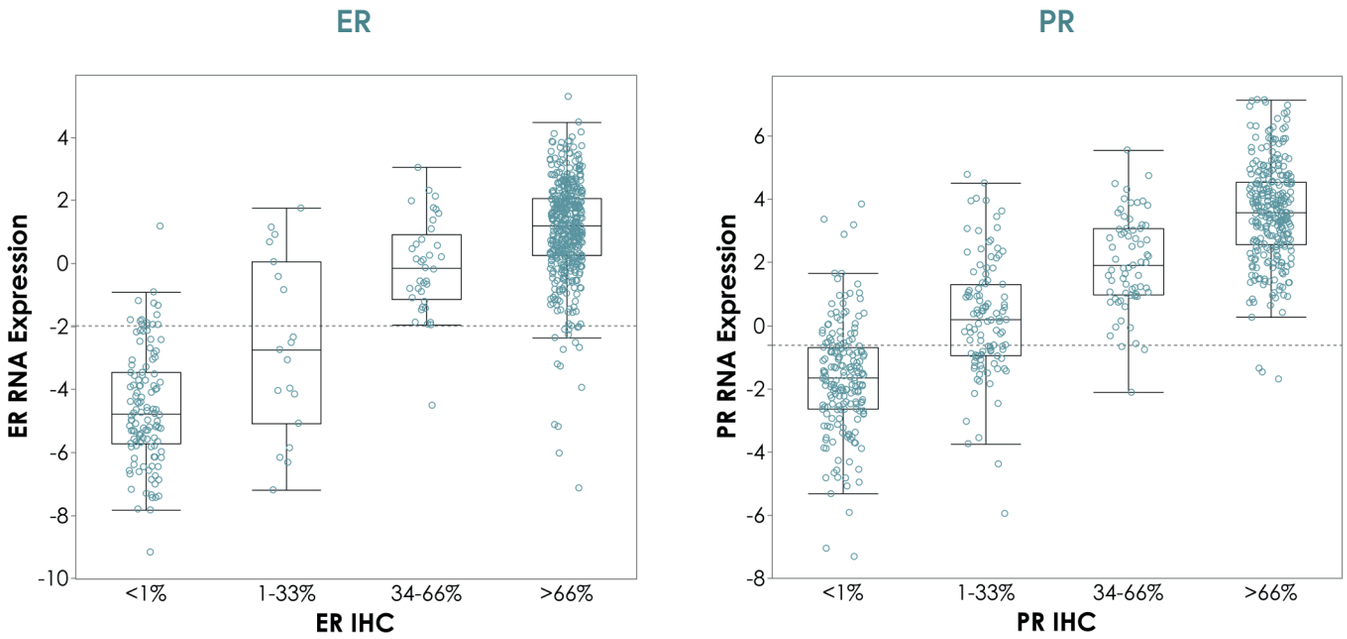
Highly Reproducible IVD with Strong Correlation to IHC

Clinical Performance Evaluation

The performance of the APIS Breast Cancer Subtyping Kit was assessed as an agreement with the current standard of care (IHC) using over 600 breast cancer CNB and resected specimens.

Accurate Detection of Hormone Receptors

Concordance for ER and PR Between APIS Breast Cancer Subtyping Kit and IHC-based Biomarker Assessment



Concordance Plots. Y axis; APIS RNA gene expression (ΔCt value) with assays cut-off (dashed line) indicating positive/negative calling.
X axis; IHC positive nuclei (%). ER and PR negative when <1%.

Highly reproducible and accurate detection of hormone receptors, ER and PR

	ER	PR
OPA	93.1% (90.9-94.8)	86.8% (84.0-89.2)
PPA	94.7% (92.5-96.4)	90.9% (88.0-93.3)
NPA	87.1% (80.5-91.7)	77.3% (71.0-82.6)

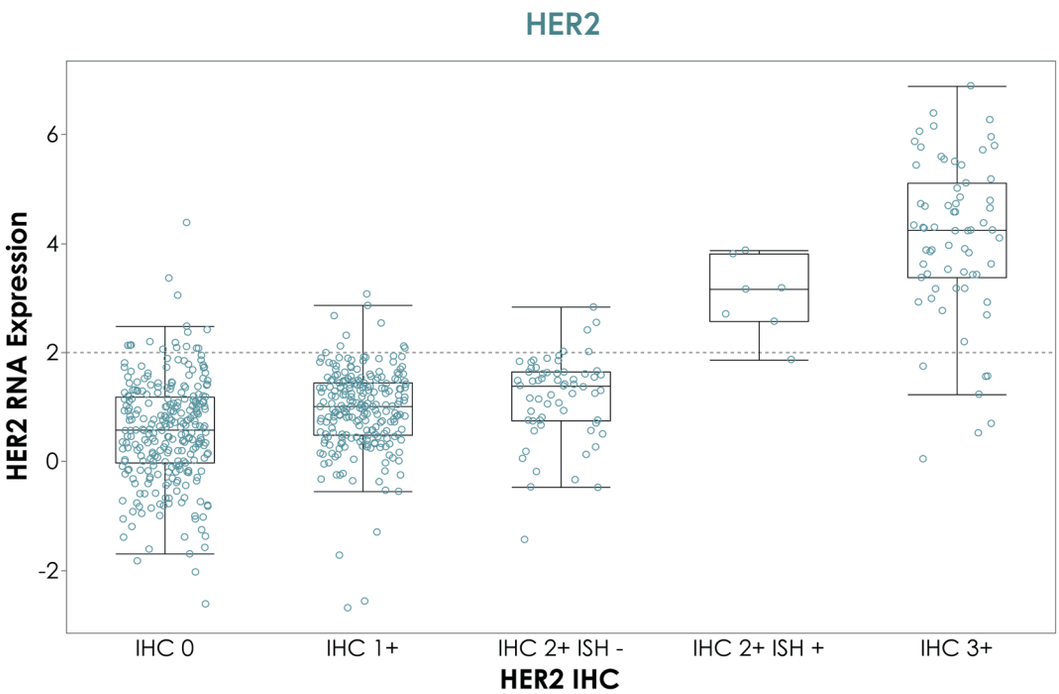
Percent Agreement with 95% Confidence Intervals.
OPA Overall percent agreement, PPA Positive percent agreement,
NPA Negative percent agreement.

Providing accurate identification of hormone receptors ER and PR with a strong correlation to IHC results, confirming assay reliability and aiding clinical decision-making.

Accurate Detection of HER2

Clinical Performance Evaluation – HER2

Concordance for HER2 Between APIS Breast Cancer Subtyping Kit and IHC-based Biomarker Assessment



Concordance Plot. HER2 scoring as per ASCO/CAP guidelines; negative (0, 1+), positive (3+), unresolved (2+) sample status assigned Positive or Negative upon reflex testing or by a pathologist prior to reflex testing. Y axis; APIS HER2 RNA expression (ΔCt value) with the cut-off for positive/negative calling set at ΔCt = 2.0. X axis; IHC status.

Providing a single resolution method for HER2 detection with the opportunity to enhance HER2 stratification

	HER2
OPA	94.2% (92.2-95.8)
PPA	89.2% (80.1-94.4)
NPA	94.9% (92.8-96.4)

Percent Agreement with 95% Confidence Intervals. OPA Overall percent agreement, PPA Positive percent agreement, NPA Negative percent agreement.

Strong correlation observed between APIS Breast Cancer Subtyping Kit results and IHC score. HER2 expression in 1+ and 0 patients, accurately detected by APIS Breast Cancer Subtyping Kit, provides potential for further HER2 stratification*.

Fast HER2 Results

With the APIS Breast Cancer Subtyping Kit, reflex testing for HER2 is no longer necessary. Our innovative approach allows for rapid HER2 results strongly correlating to IHC, whilst eliminating the need for additional testing and reducing turnaround time. This aids clinicians in making timely treatment decisions and accelerates patient care.

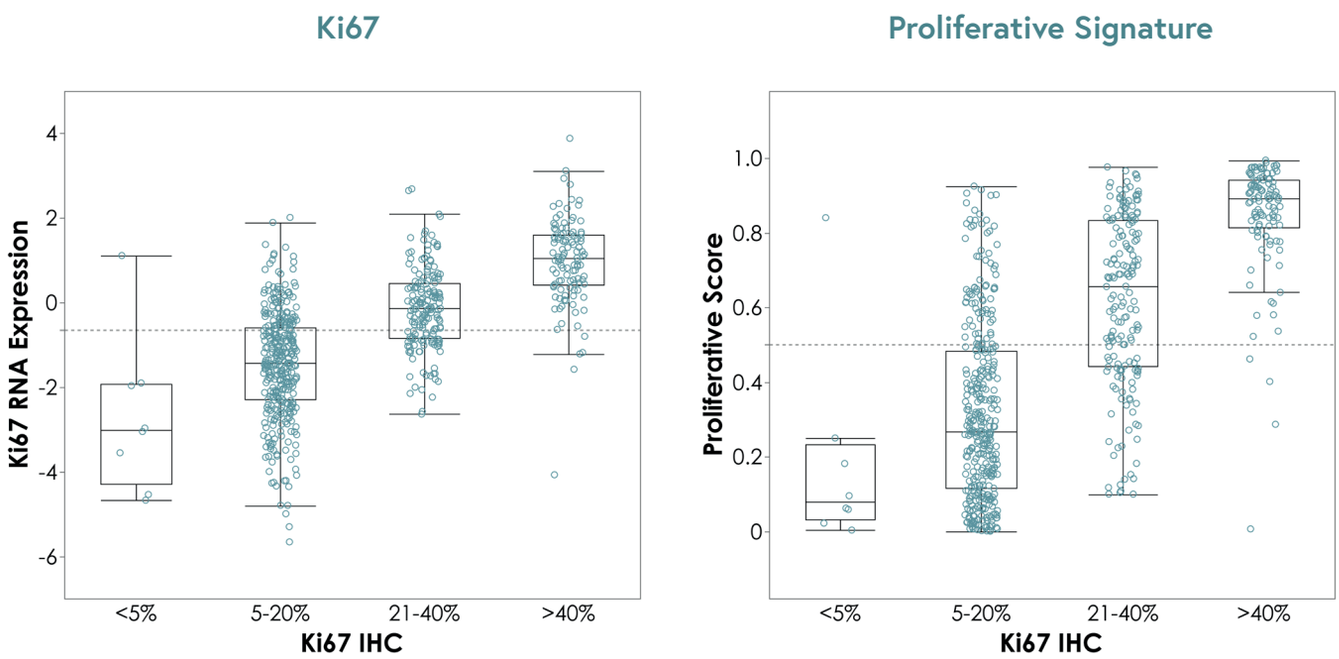
*Low HER2 validation studies and publications in progress

The Power of the Proliferative Signature

Clinical Performance Evaluation – Ki67 and Proliferative Signature

The novel APIS signature encompasses four proliferation markers (MKI67, PCNA, CCNA2 and KIF23) expressed throughout all stages of the cell cycle, enhancing the performance of Ki67 as a standalone marker.

Concordance for Ki67 and Proliferative Signature Between APIS Breast Cancer Subtyping Kit and IHC-based Biomarker Assessment



Concordance Plots. Y axis; APIS RNA gene expression (ΔCt value or proliferative score) with assays cut-off values (dashed line) for high/low calling. X axis; Semi-quantitative IHC positive nuclei %

The APIS novel four-gene proliferative signature supports the use of Ki67 alone for measuring proliferation

	Ki67	Proliferative Signature
OPA	78.3% (75.0-81.3)	80.1% (76.8-83.1)
PPA	80.3% (75.4-84.4)	79.9% (75.0-84.1)
NPA	76.6% (72.0-80.8)	80.3% (75.9-84.2)

Percent Agreement with 95% Confidence Intervals.
OPA Overall percent agreement, PPA Positive percent agreement, NPA Negative percent agreement.

Ki67 mRNA detection has a good correlation to IHC results. However, our innovative proliferative signature complements the analysis of Ki67 expression, elevating its utility beyond standalone assessments. By leveraging the power of this novel signature, our assay provides enhanced accuracy in evaluating tumour proliferation, allowing for more informed patients' decisions*.

*Validation studies in progress for the prognostic potential of the novel proliferative signature

High Reproducibility is observed for the APIS Breast Cancer Subtyping

APIS Breast Cancer Subtyping Kit Analytical Precision and Reproducibility

High precision was observed for the APIS Breast Cancer Subtyping Kit via assessment of performance attributes; including repeatability (within run precision) and reproducibility (between site precision). 3 kit lots, 4 qPCR instruments, 6 operators, 3 sites and 20 days, were used to assess reproducibility.

APIS Breast Cancer Subtyping Kit shows high reproducibility even with low target expression

Target	Between - Site	Between - Operator	Between - Instrument	Between - Lot	Between - Run (Between Day)	Within Run	Total
HER2	0.156	0.018	0.052	0.441	0.088	0.185	0.514
ER	0.000	0.338	0.000	0.495	0.229	0.331	0.722
PR	0.000	0.092	0.000	0.693	0.189	0.240	0.763
Ki67	0.060	0.099	0.070	0.405	0.098	0.279	0.520
Proliferative Signature	0.017	0.014	0.000	0.040	0.009	0.045	0.065

ΔCt values variability components attributed to each variable. Reported in terms of SD. All samples were tested at challenging low target expression levels. A total of 100 datapoints were generated per concentration per target.

The APIS Breast Cancer Subtyping Kit displays high precision and reproducibility, overcoming the observer variability encountered with immunochemistry methods

APIS Breast Cancer Subtyping Kit

Provides a **robust,** highly **reproducible** and **accurate** subtyping method

Precise

Accurate

Highly Reproducible

Easy to use

Automated Software

Innovative

Fast Results

A Molecular Profile for Breast Cancer Stratification

Accurate Detection

- Reliably detecting HER2, ER, PR & Ki67 mRNA expression levels
- Improved proliferation measure utilising a novel signature

Clinically Proven

- Backed by extensive research and clinical validation
- Reliable and efficient in accurately identifying breast cancer subtypes

Reproducible

- Highly precise, consistent results across multiple samples
- Overcoming the observer variability encountered with IHC methods
- In-house testing for any professional molecular biology laboratory

Fast Results

- Single resolution method for determining HER2 status
- Quick result calling with automated software
- Performed and analysed in <5 hours

Diagnostic Utility

- Supporting clinicians in tailoring treatment strategies
- Reliable and strong correlation to IHC
- Streamlining the diagnostic process

Ordering Information

Product Name	Test Type	Kit Size	Catalogue Number	Price
APIS Breast Cancer Subtyping Kit	UKCA	24 Tests	0010	Available upon request

The APIS Breast Cancer Subtyping Kit is available as a UKCA marked product.

All scientific, analytical, and clinical information in this brochure comes from the APIS Breast Cancer Subtyping Kit Instructions for Use ART0030.

APIS
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To order the APIS Breast Cancer Subtyping Kit or to learn more about how our assay can elevate your breast cancer diagnostic capabilities, please contact your local distributor using the details below.

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