APIS
Assay Technologies Ltd.

APIS Breast Cancer Subtyping Kit

Understanding the Proliferative Signature

Technical Sheet

Tumour cell proliferative activity is an important prognostic and predictive factor in breast cancer[1]. The APIS Breast Cancer Subtyping Kit evaluates proliferation by detecting Ki67 mRNA expression and utilising a novel proliferative signature in pre-operative core-needle biopsy (CNB) or resected formalin-fixed paraffin-embedded (FFPE) breast tumour tissue.

Comprehensive Proliferation Assessment

Ki67 mRNA detection

- The kit is optimised for the sensitive and specific detection of Ki67 mRNA, a well-established marker for assessing proliferative activity in tumour cells
- Enables accurate quantification of Ki67 expression levels, aiding in the determination of tumour aggressiveness and prognosis
- Ki67 mRNA expression is known to be more robust than immunohistochemistry (IHC) which faces challenges in standardisation and inter-laboratory variability[1]

Novel Proliferative Signature

- Incorporates a novel proliferation measure that extends beyond Ki67, providing a comprehensive assessment of tumour cell proliferation
- The signature is designed to capture additional molecular markers associated with cell cycle progression, enhancing the subtyping capabilities of the kit

Proliferation Markers

- ***MKI67*** – Proliferation Marker Protein Ki67 - widely used marker for proliferation, associated with poor prognosis[2]
- ***CCNA2*** – Cyclin A2 - associated with poor prognosis and worse overall survival[3,4]
- ***KIF23*** – Kinesin Family member 23 - associated with poor prognosis, lymph node invasion, and tumour recurrence[5,6]
- ***PCNA*** – Proliferating cell nuclear antigen - associated with poor prognosis and poor overall survival[7]

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Proliferation Markers in the Cell Cycle

The cell cycle describes the processes that occur within a cell during duplication of its DNA to produce two cells. It consists of cell division and the distinct phases, including interphase (G1, S, and G2 phases) and mitosis (M phase).

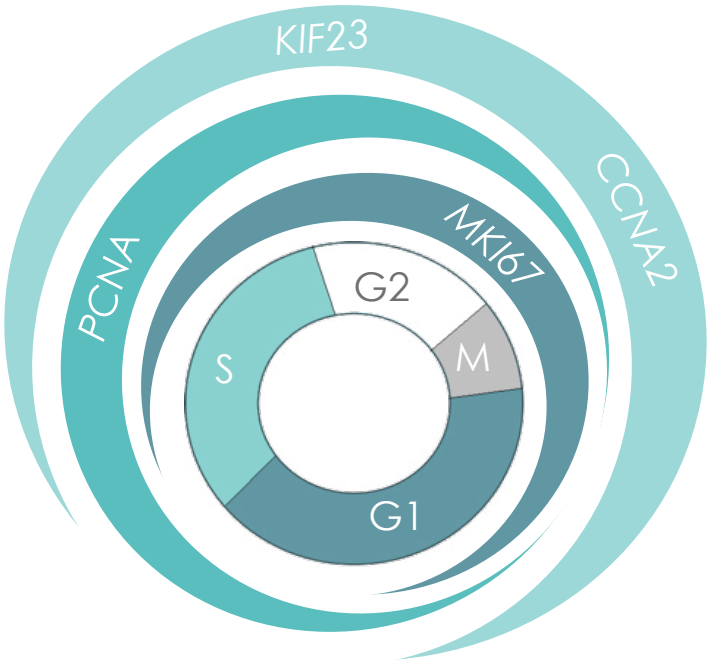


Figure 1. Proliferation marker expression within the cell cycle. Increased curve thickness indicates higher levels of expression.

Table 1. Peak expression of proliferation markers in cell cycle phases.

Cell Cycle Expression		
Gene	**Peak expression**	**Other**
CCNA2	G2	G1/M
KIF23	G2	G2/M, M
PCNA	G1/S	S
MKI67	G2/M	M

- Our multi-gene approach offers a holistic view of cell proliferation dynamics
- Collectively the score indicates the rate at which cells are actively dividing or proliferating

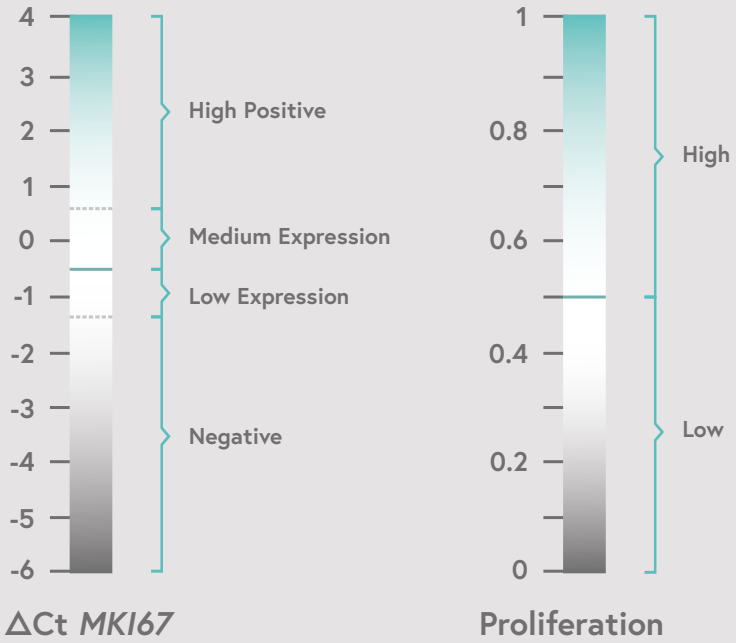
Exploring distinctions between *MKI67* and proliferative signature assessments

Discrepancies between proliferative and *MKI67* measurements may arise, these reflect aspects of tumour biology and may include the following:

- *MKI67* RNA expression indicates the proportion of cells actively progressing through the cell cycle at a given moment. As tumour cells may be at different phases of the cell cycle, the expression levels of *MKI67* may vary.
- The results provided by the proliferative signature are based on the expression of multiple markers throughout the cell cycle (Figure 1), reflecting a more rounded measure of proliferation than *MKI67* alone. Therefore, the results of these two measures may not completely align.
- For example, *MKI67* expression does not always correlate with overall proliferation due to the cell cycle not being synchronised across the tumour[8]. The additional information provided by the proliferative signature could enhance subtyping capabilities.

Interpreting *MKI67* and proliferation together

- Where proliferation and *MKI67* do not agree the output should be assessed using a semi-quantitative approach
- *MKI67* calls falling within low to medium expression ΔCt (-1.26 - 0.42) are subject to variation in expression across the tumour due to tumour heterogeneity or "hot spot" expression
- Assessing the proliferative signature in tumours with low to medium *MKI67* expression can aid in calling high or low proliferation
- Interpretation should always include histopathology and other clinical features
- If results strongly disagree further testing may be warranted



References

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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 research capabilities, please contact your local distributor using the details below.

UK sales agent:

LINK medical

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Phone: +44 (0) 203 1373 193
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Ordering Information

Product Name	Test Type	Kit Size	Catalogue Number	Price
APIS Breast Cancer Subtyping Kit	IVD	24 Samples in duplicate plus controls	00102 (distributed by APIS)	Available upon request
APIS Breast Cancer Subtyping Kit	RUO	24 Samples in duplicate plus controls	00402 (distributed by Biocartis)	Available upon request
APIS Breast Cancer Subtyping Kit	RUO	24 Samples in duplicate plus controls	00403 (distributed by APIS)	Available upon request

Please visit the RUO product web page for the list of countries that Biocartis distributes the APIS Breast Cancer Subtyping Kit. APIS is the distributor for all other countries.

The Research Use Only (RUO) Kit is not for use in diagnostic procedures.
For use in diagnostic procedures, the IVD Kit catalogue number is required.