APIS
Assay Technologies Ltd.

APIS Breast Cancer Subtyping Kit

Integrating RT-qPCR and IHC: Result Interpretation

Technical Sheet

The APIS Breast Cancer Subtyping Kit is a highly reproducible, RNA-based diagnostic workflow for detecting mRNA expression of ER, PR, HER2, and Ki67. The APIS Breast Cancer Subtyping Kit Analysis Software automates results interpretation, calling target expression as high/positive or low/negative. This resource aims to provide the user with key insights into the comparison of immunohistochemistry (IHC) and mRNA-based analyses in the context of the APIS Breast Cancer Subtyping Kit output.

APIS Breast Cancer Subtyping Kit Report Output

Binary marker status validated against IHC

Positive or Negative

- *ESR1*/ER (oestrogen receptor 1)
- *PGR*/PR (progesterone receptor)
- *ERBB2*/HER2 (human epidermal growth factor receptor 2)

High or Low

- *MKI67*/Ki67 (marker of proliferation Ki-67)
- Proliferation (proliferative signature)

Molecular subtype is constructed as per the St Gallen guidelines[1]

- Luminal A
- Luminal B (HER2 negative)
- Luminal B (HER2 positive)
- HER2 enriched (non-luminal)
- Triple negative

Marker status table from APIS Breast Cancer Subtyping Kit software

Indicating a qualitative call for all targets. RNA expression results are reported as ΔCt, where the target Ct value is normalised to the assay reference genes.

Table 1. Kit analysis software example results. Molecular subtype, marker status and associated ΔCt are automatically reported.

Marker	Status	ΔCt
ESR1	Negative	-3.94
PGR	Negative	-1.14
ERBB2	Positive	2.61
MKI67	High	0.28
Proliferation	High	0.91
Marker	**HER2 enriched**	

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Alignment of ΔCt with Immunohistochemistry

IHC scores were correlated with ΔCt values and validated using RNA copy numbers as determined by digital PCR (dPCR).

For each target, ΔCt ranges were established, facilitating classification into negative, low, medium, and high expression.

ER/*ESR1*

IHC is scored using the Allred or Immunoreactivity (IRS) score[2].

Table 2. Interpretation of ER/*ESR1* expression with IHC scoring or APIS Breast Cancer Subtyping Kit (RT-qPCR) using ΔCt.

Allred Score	IRS Score	IHC Interpretation	RT-qPCR range *ESR1* ΔCt	RT-qPCR Interpretation
7-8	9-12	Receptor positive, strong expression	> 1.22	High Positive
5-6	4-8	Receptor positive, moderate expression	> 0.11 - ≤ 1.22	Moderate Positive
3-4	2-3	Receptor positive, weak expression, Low ER	> -1.98 - ≤ 0.11	Low Positive
0-2	0-1	Receptor negative	≤ -1.98	Negative

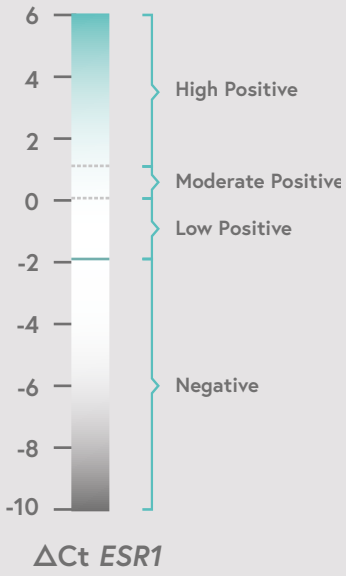

Figure 1. ΔCt scale for *ESR1* expression

PR/*PGR*

IHC is scored using the Allred or Immunoreactivity score[2].

Table 3. Interpretation of PR/*PGR* expression with IHC scoring or APIS Breast Cancer Subtyping Kit (RT-qPCR) using ΔCt.

Allred Score	IRS Score	IHC Interpretation	RT-qPCR range *PGR* ΔCt	RT-qPCR Interpretation
7-8	9-12	Receptor positive, strong expression	> 2.87	High Positive
5-6	4-8	Receptor positive, moderate expression	> 1.21 - ≤ 2.87	Moderate Positive
3-4	2-3	Receptor positive, weak expression	> -0.63 - ≤ 1.21	Low Positive
0-2	0-1	Receptor negative	≤ -0.63	Negative

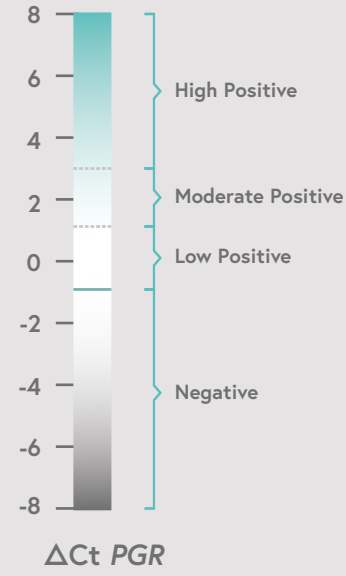

Figure 2. ΔCt scale for *PGR* expression

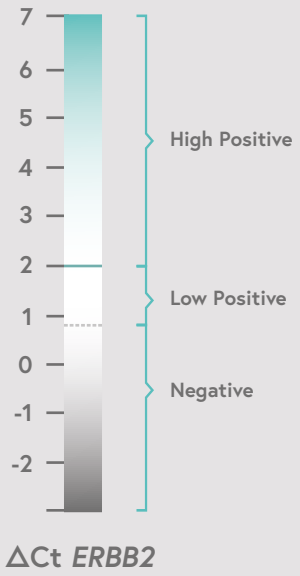

Figure 3. ΔCt scale for *ERBB2* expression

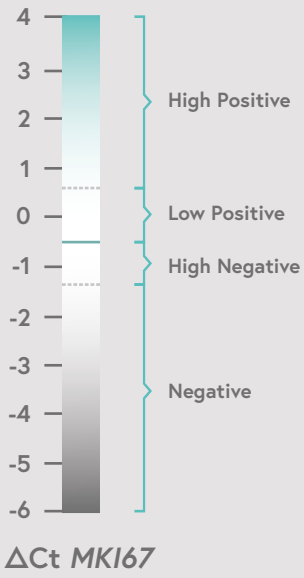

Figure 4. ΔCt scale for *MKI67* expression

HER2/*ERBB2*

HER2 IHC/ISH is a semi-quantitative system based on the intensity of reaction staining and the percentage of membrane-positive cells followed by ISH for reflex testing of 2+ tumours[3].

Table 4. Interpretation of HER2/*ERBB2* expression with IHC/ISH or APIS Breast Cancer Subtyping Kit (RT-qPCR) using ΔCt.

HER2 Score	IHC Interpretation	RT-qPCR range *ERBB2* ΔCt	RT-qPCR Interpretation
3+	Receptor positive	> 2.0	High Positive
2+ (ISH amplified)	Receptor positive		
2+ (ISH non-amplified)	Receptor negative, moderate HER2 (HER2 – Low)	> 0.70 - ≤ 2.0	Low Positive (HER2-low)
1+	Receptor negative (HER2 - Low)		
0	Receptor negative	≤ 0.70	Negative

Ki67/*MKI67*

Ki67 IHC is a semi-quantitative system based on the percentage of stained cells found within the section, with staining below 20% representing a low proliferating tumour[4].

Table 5. Interpretation of Ki67/*MKI67* expression with IHC or APIS Breast Cancer Subtyping Kit (RT-qPCR) using ΔCt.

Ki67 % Staining	IHC Interpretation	RT-qPCR range *MKI67* ΔCt	RT-qPCR Interpretation
>30%	High expression	> 0.42	High Positive
21-30%	Moderate expression	> -0.64- ≤ 0.42	Low Positive
10-20%	Low expression	> -1.26- ≤ -0.64	High Negative
<10%	Very low expression	≤ -1.26	Negative

The established RT-qPCR ΔCt cut-off points provide a semi-quantitative scale for evaluating targets with the APIS Breast Cancer Subtyping Kit, aligning with current IHC outputs.

References

1 Burstein HJ, et al. Ann Oncol. 2021 Oct;32(10):1216-1235.
2 Allison KH, et al. J Clin Oncol. 2020 Apr 20;38(12):1346-1366.
3 Wolff AC, et al. J Clin Oncol. 2023 Aug 1;41(22):3867-3872.
4 Nielsen TO, et al. J Natl Cancer Inst. 2021 Jul 1;113(7):808-819.

APIS
Assay Technologies Ltd.

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

LINK Medical Solutions

Phone: +44 (0) 203 1373 193
Address: 85 Great Portland St, First Floor, London, W1W 7LT
Email: info@linkmedicalsolutions.com

International distributor:

Distributed by BIOCARTIS

Biocartis

Phone: +32 (0) 15 632 600
Address: Generaal De Wittelaan 11B, 2800 Mechelen, Belgium
Email: info@biocartis.com

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.