

Cepheid® ONCore Report

The Xpert® Breast Cancer STRAT4 assay (STRAT4)¹ measures the gene expression (mRNA) levels of 4 breast cancer biomarkers. Individual biomarker STRAT4 gene expression scores are determined by the delta Ct (dCt). The dCt value is calculated as the difference between the reference gene Ct and target biomarker Ct values. The more positive the score, the higher the expression. STRAT4 cutoffs for individual biomarkers were validated¹ based on concordance with IHC (or HER2 FISH, when applicable) results according to ASCO/CAP^{2,3} and ESMO⁴ guidelines. The STRAT4 score for each biomarker is individually plotted on a graphical scale that displays the positive and negative reference range and numerical cut-off value for that biomarker in the assay. In some cases, where no biomarker amplification is detected, the GeneXpert® Dx software may show no score. In these cases, the scores are displayed as -10 and are represented on this graph as ≤ -10 . An indeterminate value is given when expression levels cannot be determined due to sample containing insufficient material.

ESR1 is the gene encoding the Estrogen Receptor (ER). ESR1 cutoffs for the STRAT4 assay were validated relative to ER IHC results using the 6F11 antibody clone with a cutoff for positivity of $\geq 1\%$ invasive tumor cells showing definite nuclear staining, irrespective of staining intensity.

PGR is the gene encoding the Progesterone Receptor (PR). PGR cutoffs for the STRAT4 assay were validated relative to PGR IHC results using the PgR636 antibody clone with a cutoff for positivity of $\geq 1\%$ invasive tumor cells showing definite nuclear staining, irrespective of staining intensity.

ERBB2 is the gene encoding the Human Epidermal Growth Factor Receptor 2 (HER2). ERBB2 results by the STRAT4 assay were validated relative to HER2 IHC and FISH, with HER2 positive results defined as IHC 3+ and/or HER2 FISH amplified (defined as HER2:CEP17 ratio ≥ 2.0 and/or average HER2 copy number ≥ 6.0 signals/cell).

MKi67 is the gene encoding for the marker of proliferation, Ki-67. MKi67 cutoffs for the STRAT4 assay were validated relative to Ki67 IHC using the MIB-1 antibody with a cutoff for positivity of $\geq 20\%$ of invasive tumor cells showing definite nuclear staining, irrespective of staining intensity.

References:

1. Xpert Breast Cancer STRAT4 CE-IVD Package Insert.
2. *ASCO-CAP Guideline Recommendations for IHC testing of ER/PR in Breast Cancer: Hammond et al. (2010) JCO 28;16; 2784-2795.*
3. *ASCO-CAP Guideline Recommendations for HER2 Testing in Breast Cancer: Wolff et al. (2013) JCO 31;31; 3997-4013.*
4. *Primary Breast Cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up: Senkus et al (2015) Annals of Oncology supplement 5; v8-30.*

¹ CE-IVD. *In Vitro* Diagnostic Medical Device.