

# BREAST CANCER PROGRAMMES

## Recommended Internal Quality Control Tissues

Choosing appropriate control tissues is vitally important in enabling you to critically assess the quality of your immunocytochemical staining. This is particularly true when using prognostic and predictive markers that require some degree of quantitative interpretation.

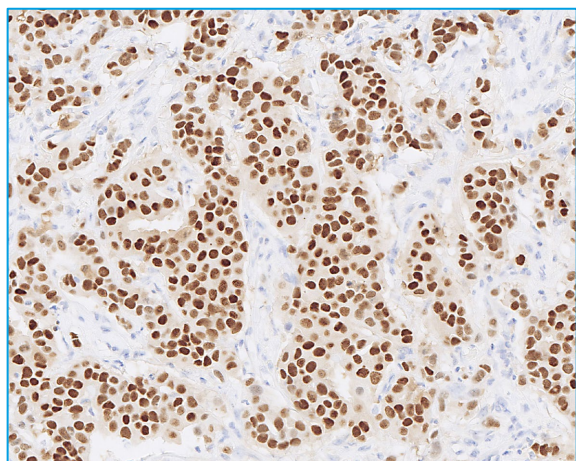
We have produced this guide to aid you in choosing the correct controls for the markers used in our Breast EQA Programmes and have illustrated the expected staining results for those controls.

We have confined our selections to tissue specimens that you should be able to find in your own archives, but we also recognise that commercially available cell-lines are useful additional controls.

### Oestrogen Receptors

Oestrogen receptor (ER) protein is expressed in a variety of tissue types including breast, where it is found in a proportion of the epithelial cells lining the ducts. It is a nuclear receptor which binds the steroid hormone, oestrogen. Overexpression of ER is seen in approximately 80% of breast cancers where it is a key driver of tumour cell proliferation.

The correct identification of ER-positive cancer is extremely important as it is amenable to treatment using endocrine therapies, which offer little or no benefit in ER-negative disease.



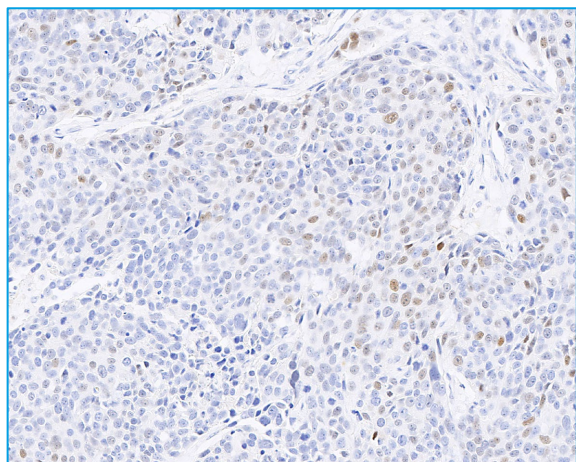
#### Breast cancer demonstrating strong ER expression

The majority (>90%) of the tumour cells in this case are expressing ER at strong or moderate intensity (Allred score of 7 or 8, H-score in the region of 220 – 240).

The staining is for the most part confined to the tumour nuclei although the cytoplasm of the tumour cells also show staining at a very low intensity, which is a usual finding.

In contrast, in the interdigitating areas of connective tissue the stromal cells are completely unstained.

(Original objective magnification x20)



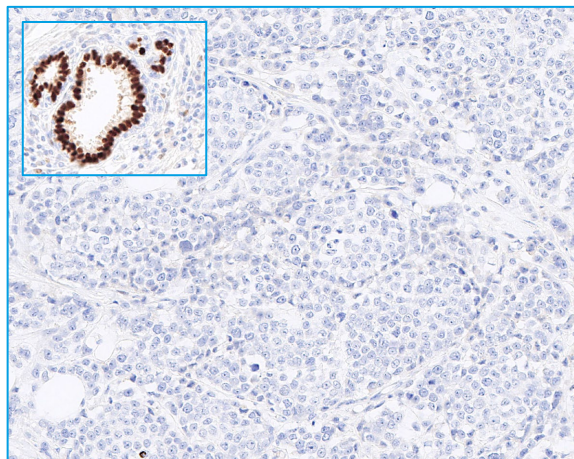
#### Breast cancer demonstrating weak ER expression

Approximately 20% of the tumour cells are expressing detectable signal at a weak or occasionally moderate intensity (Allred score of 4, H-score in the region of 20 – 30). This would still be considered to be an ER-positive case, with expression at the lower end of the positive spectrum.

The level of the counterstain allows identification of the ER-negative nuclei but does not mask the delicate signal in the ER-positive cells.

This is an ideal control, capable of rigorously testing the sensitivity of the test method. A small decline in sensitivity would cause many of the weakly stained tumour cells to become unstained.

(Original objective magnification x20)

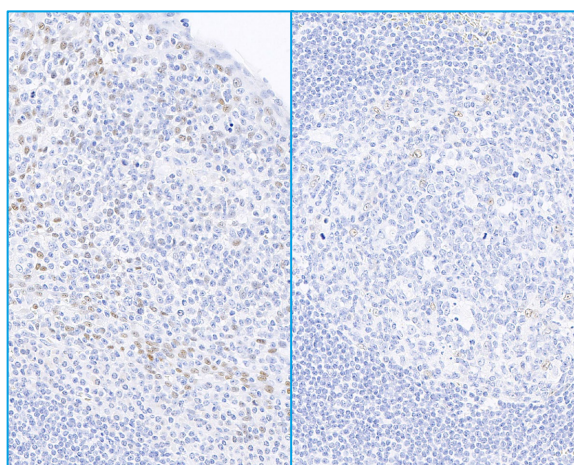


## Breast cancer lacking demonstrable ER expression

None of the tumour cells in this case are expressing ER (Allred score of 0, H-score of 0).

When choosing an ER-negative case for use as quality control tissue it is preferable to include an area of uninvolved tissue which contains ER expressing normal duct cells. This ensures the lack of staining in the tumour has not been caused by sub-optimal pre-analytical conditions e.g., delayed or excessively prolonged fixation. In this case an area of normal duct cell staining from elsewhere in the specimen is shown in the inset at the top-left of the image.

(Original objective magnification x20)



## Reactive tonsil

Non-tumour ('normal') tissues that express the target antigen at low levels and/or at levels close to clinical cut-offs make excellent sensitivity controls because they reproducibly express the antigen at similar levels from one sample to another.

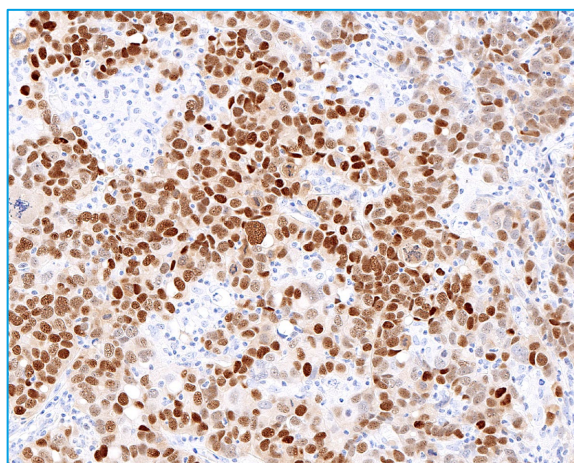
The crypt epithelium in reactive tonsil shows weak ER staining in 10 – 20% of its cells (see left-hand panel), and a small number of cells (<1%) found in reactive follicle centres will staining very weakly (right-hand panel). Both these percentages will vary according to the primary antibody clone used. Elsewhere in the mantle zones and inter-follicular areas no ER expression is seen.

(Original objective magnification x20)

# Progesterone Receptors

Progesterone receptor (PgR) gene expression is modulated by ER signalling, and for this reason PgR-positive ER-negative breast tumours are rare. In ER-positive breast cancer high levels of PgR are associated with less aggressive tumours, and hence it is an important prognostic indicator.

Like ER it is expressed in a variety of tissue types in addition to the breast, e.g., uterus, ovary and prostate. It is a nuclear receptor which binds the steroid hormone, progesterone. When PgR is over-expressed it tends to be present at high levels and it can be quite difficult to find cases showing weak or moderate expression.



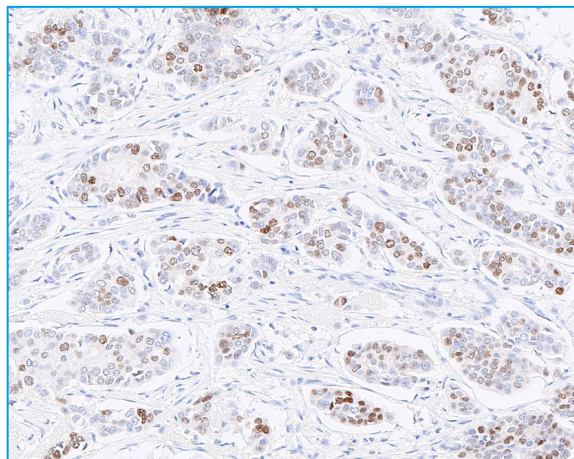
## Breast cancer demonstrating strong expression of PgR

Approximately 60% of the tumour cells in this case are expressing PgR at moderate intensity with a further 20 – 30% showing strong expression (Allred score of 7, H-score in the region of 180 – 210).

In a similar way to ER, staining is for the most part confined to the tumour nuclei although the cytoplasm of the tumour cells also show staining at a very low intensity, which is a usual finding.

In the connective tissue the stromal cells are completely unstained.

(Original objective magnification x20)



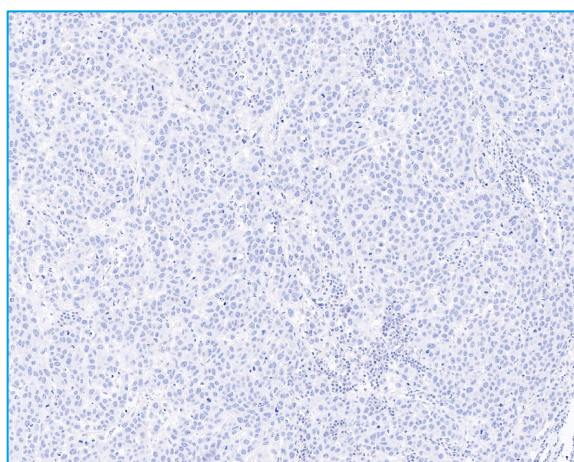
## Breast cancer showing weak or moderate PgR expression

Approximately 50% of the tumour cells are expressing detectable signal at a weak (20%) or moderate (30%) intensity (Allred score of 6, H-score of 80). This would be considered as an PgR-positive case, with expression in the mid-range of the positive spectrum.

The level of the counterstain allows identification of the ER-negative nuclei but does not mask the delicate signal in the ER-positive cells.

A control showing this level of staining will test the sensitivity of the test method since a small decline in sensitivity would cause the weakly stained tumour cells to become unstained.

(Original objective magnification x20)

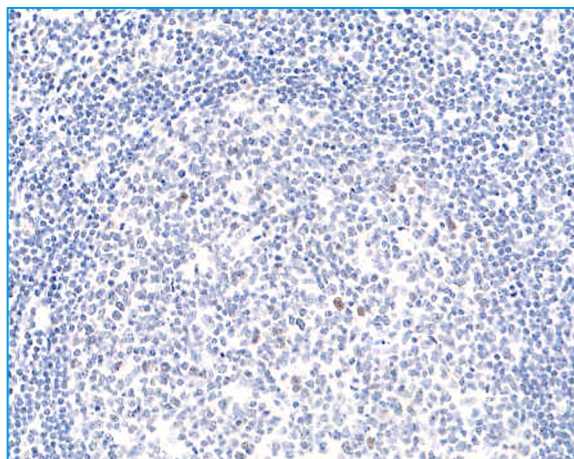


## Breast cancer lacking demonstrable PgR expression

None of the tumour cells in this case express PgR (Allred score of 0, H-score of 0).

While it is preferable when choosing a PgR-negative case for use as quality control tissue to include an area of uninvolved tissue which contains PgR expressing normal duct cells, it is not always possible. This is because PgR is variably expressed in negative ducts and can be absent.

(Original objective magnification x20)



## Reactive tonsil

The usefulness of tonsil as a normal tissue control for PgR staining is dependent on the primary antibody clone. Many of the commonly used clones will show no staining in tonsil.

In contrast the 1E2 clone does produce weak staining in <3% of nuclei in the cells found in follicle centres.

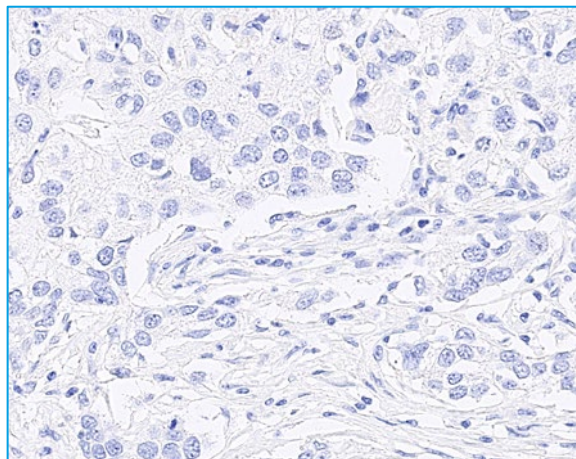
In specimens stained with 1E2, the crypt epithelium usually shows only occasional weakly positive cells, and it is more commonly completely unstained.

(Original objective magnification x40)

## Human epidermal growth factor receptor 2

Amplification of the human epidermal growth factor receptor 2 (HER2) gene is known to be the key oncogenic driver in 15 - 20% of all breast cancer cases. Amplification of the gene leads to over-expression of HER2 protein in tumour cells. HER2-positive breast cancer has a decreased overall survival due to its association with an aggressive phenotype and increased recurrence rate.

HER2-positive breast cancer, defined as that showing 3+ staining by immunocytochemistry or 2+ staining with gene amplification confirmed by *in-situ* hybridisation can be treated using humanised monoclonal antibodies targeting the HER2 protein (e.g., trastuzumab). Recently antibody-drug conjugates (e.g., trastuzumab deruxtecan) have been introduced that are effective in the treatment of HER2-low (1+ and 2+ non-amplified) disease.



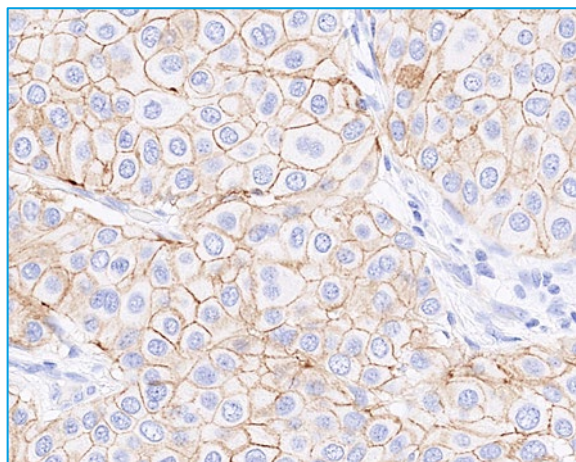
### Breast cancer showing HER2 0 staining

This breast tumour lacks any demonstrable HER2 protein.

The counterstain allows identification of the HER2-negative tumour but does not mask any delicate HER2 signal which may be present.

It is advisable to include a HER2-negative case in your control set primarily as it will be a sensitive indicator of non-specific staining.

(Original objective magnification x40)



### Breast cancer showing HER2 2+ staining

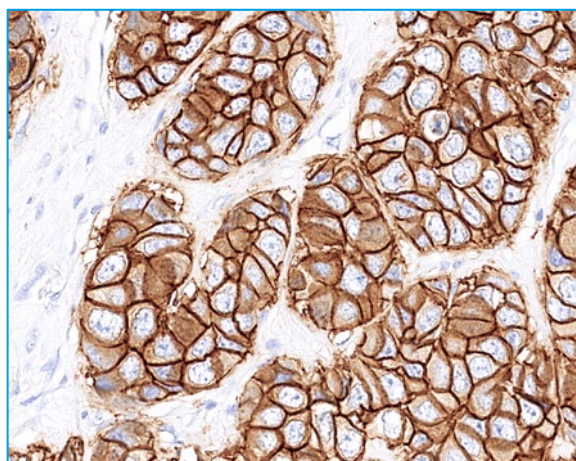
HER2 2+ staining is defined as invasive breast cancer with complete (circumferential) membrane staining of weak to moderate intensity observed in >10% of tumour cells (ASCO/CAP guidelines).

The UK recommendations add the staining pattern of invasive breast cancer with strong or moderate membrane staining intensity in ≤10% of tumour cells.

The illustrated example shows moderate complete membrane staining in >10% of tumour cells.

The stromal cells lack any staining.

(Original objective magnification x40)



### Breast cancer showing HER 3+ staining

The ASCO/CAP guidelines and the UK recommendations define HER2 3+ staining as circumferential membrane staining that is complete, intense and in >10% of invasive tumour cells.

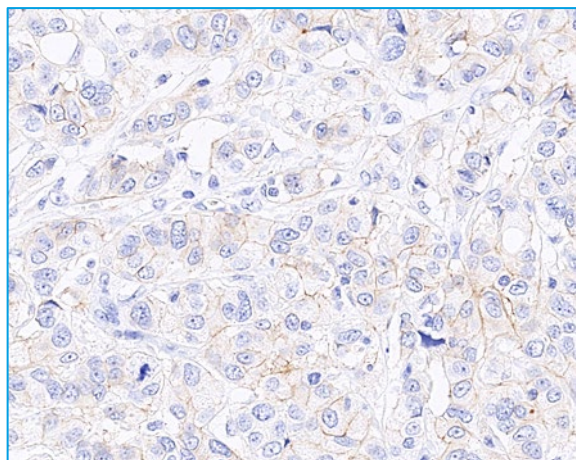
In the tumour shown here the membranes of almost all invasive cells are intensely stained.

It is important not to rely on the use of a HER2 3+ control alone as such a tissue will not be able to detect any changes in the sensitivity of the IHC method that may occur from run to run.

(Original objective magnification x40)

## HER2-Low

When carrying out staining that is intended for use in detecting HER2-low disease it is recommended to include a control showing HER2 1+ staining. The ideal control tissue set would also include HER2 0 and HER2 2+ samples.



### Breast cancer showing HER2 1+ staining

The currently accepted definitions of HER2 1+ expression are:

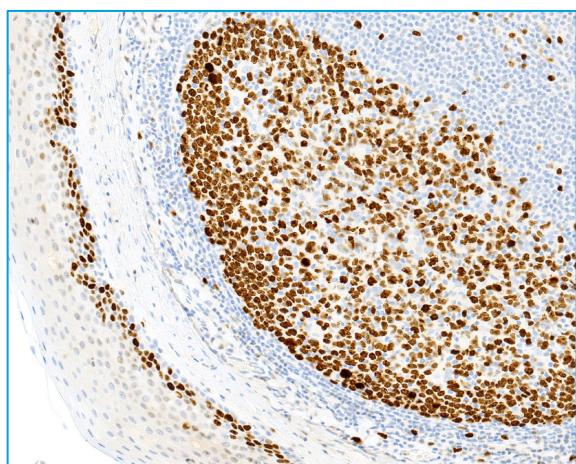
- Incomplete membrane staining that is faint/barely perceptible and in >10% of invasive tumour cells (ASCO/CAP).
- Weak complete membrane staining in ≤10%, or weak incomplete membrane staining in >10%, or faint/barely perceptible membrane staining in >10% of invasive tumour cells (UK recommendations).

The image illustrates a tumour with staining that complies with these definitions. The counterstain allows identification of the tumour but does not mask any delicate HER2 signal which may be present.

(Original objective magnification x40)

## Ki-67

Ki-67 is a marker of proliferating cells i.e., ones that are not in the resting phase ( $G_0$ ) of the cell cycle. It is a nuclear protein. In breast cancer it is used as a prognostic biomarker and as a predictor of response to chemotherapy. Various cut-offs are in use with perhaps the most commonly ones being 10%, with tumours expressing Ki-67 in <10% of their cells being regarded as low-risk, and ≥20% which is associated with tumours having a more aggressive phenotype.



### Reactive tonsil

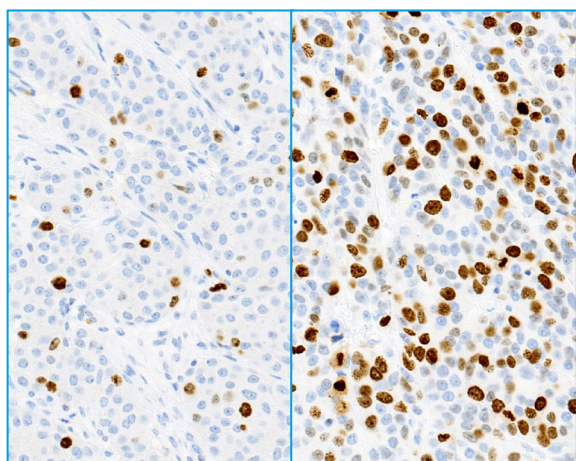
Tonsil is a very useful control tissue.

The proliferation rate in reactive follicle centres is consistently around 85%, while in the mantle zones and inter-follicular areas it is closer to 5% or less.

If squamous epithelium is present the basal layers of cells will show high (>70%) of the Ki-67 protein, with a high intensity. Staining intensity and proliferation rate is much less in the more superficial layers.

When assessing a tissue any degree of positivity within invasive tumour nuclei, no matter how weak should be regarded as positive.

(Original objective magnification x20)



### Breast cancer samples showing two different Ki-67 expression rates

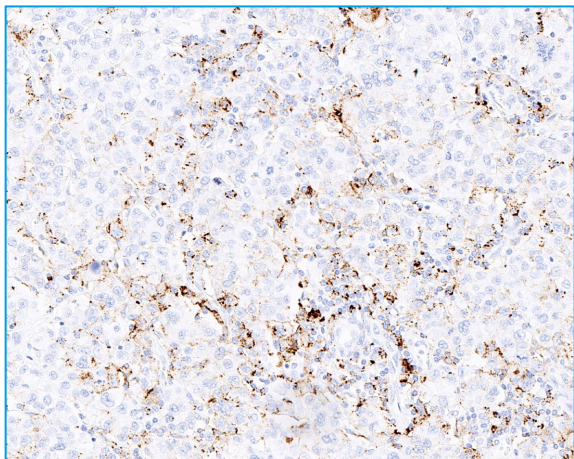
It is recommended to include two breast cancer specimens, one showing a low proliferation rate e.g., 5% (as is illustrated in the left-hand panel), and one showing a high proliferation rate e.g., 20 – 30% (as is seen in the tumour illustrated in the right-hand panel).

Achieving the correct level of the nuclear counterstain is particularly important when assessing Ki-67 because as has already been mentioned, any degree of nuclear signal is regarded as indicating positivity and therefore it is possible to obscure the Ki-67 staining in very weakly stained positive nuclei if the counterstain is too intense.

(Original objective magnification x40)

## PD-L1 in Triple Negative Breast Cancer

The assessment of PD-L1 staining is required to determine patients' eligibility for immunotherapy. In Triple Negative Breast Cancer (TNBC) two different primary antibodies are used in the companion diagnostic assays approved for use in predicting response, these are SP142 and 22C3. Which one is used depends upon the nature of the immunotherapy the clinician intends to prescribe. The staining patterns SP142 and 22C3 produce are very different from one another, requiring specifically designed scoring methods for their assessment.

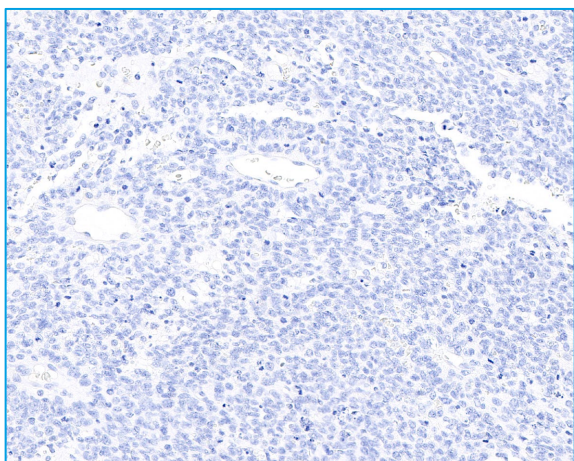


### PD-L1 positive breast cancer sample (SP142)

SP142 staining is assessed using the Immune Cell (IC) scoring system (assesses staining in lymphocytes and macrophages). The result is expressed as the percentage of the tumor area occupied by PD-L1 positive immune cells; in TNBC the positive cut-off is  $\geq 1\%$ .

In this positive control large numbers immune cells show strong membrane expression of the antigen. The staining has a characteristic punctate appearance.

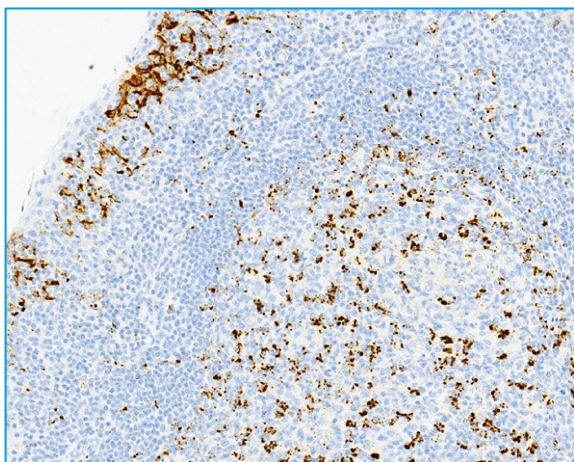
(Original objective magnification x40)



### PD-L1 negative breast cancer sample (SP142)

It is recommended to include a PD-L1 negative breast tumour such as the one illustrated here. This will show any non-specific staining which may introduced due to changes in IHC staining reagents or changes in reagent performance due to batch-to-batch variations.

(Original objective magnification x40)

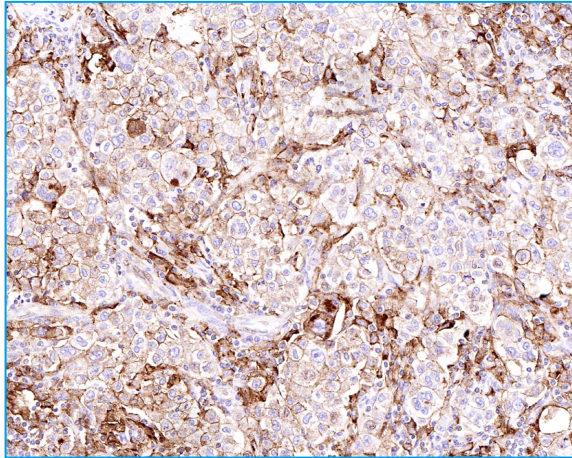


### Tonsil specimen (SP142)

The SP142 clone should stain crypt epithelium in the tonsil homogenously with a moderate to strong intensity. Scattered cells in the inter-follicular areas will show positivity.

In reactive follicle centres large numbers of cells (mainly 'starry sky' macrophages) will show intense punctate membrane staining.

(Original objective magnification x20)

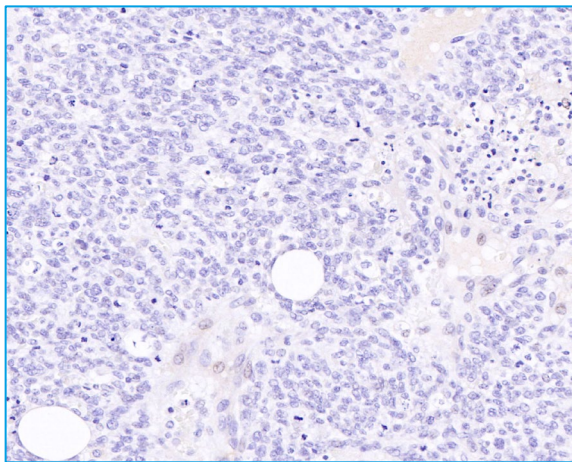


## PD-L1 positive breast cancer sample (22C3)

IHC staining in TNBC with the primary antibody clone 22C3 is undertaken using the Combined Positive Score (CPS), which is calculated as the number of PD-L1 staining cells (invasive tumour cells, lymphocytes, macrophages) divided by the total number of invasive tumour cells, multiplied by 100. A CPS of  $\geq 10$  indicates the patient is eligible for immunotherapy.

In this positive control the majority of tumour and immune cells are positively stained. In the tumour cells the staining is clearly seen to be membrane-located.

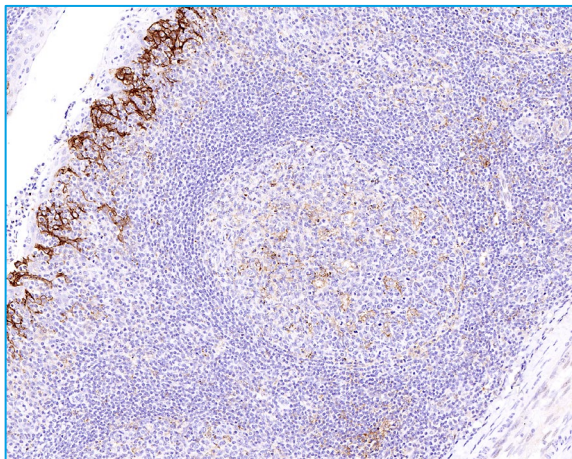
(Original objective magnification x40)



## PD-L1 negative breast cancer sample (22C3)

In this PD-L1 negative tumour there is no staining in the tumour or tumour associated immune cells.

(Original objective magnification x40)



## Tonsil specimen

The 22C3 primary antibody clone should stain crypt epithelium in the tonsil homogeneously with a moderate to strong intensity in a similar way to SP142. Scattered cells in the inter-follicular areas will show positivity.

In reactive follicle centres large numbers of cells (mainly 'starry sky' macrophages) will show staining but here the appearance of the reaction product differs from that seen in SP142 staining; it is much less intense and is more linearly delineated along cell membranes.

(Original objective magnification x20)