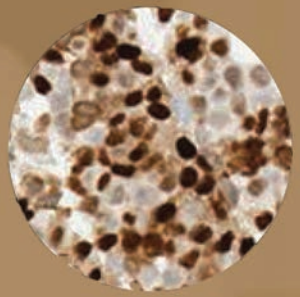
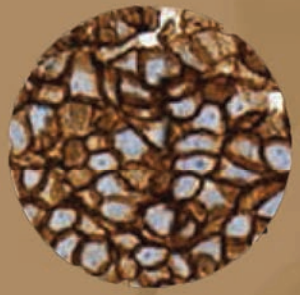
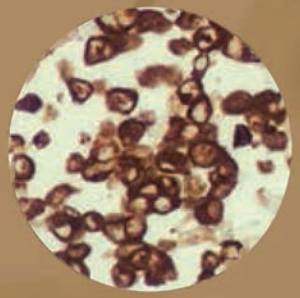
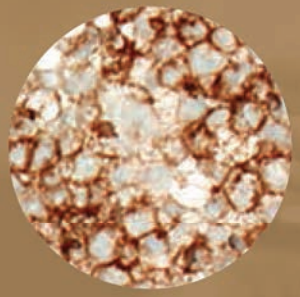








HistoCyte Laboratories

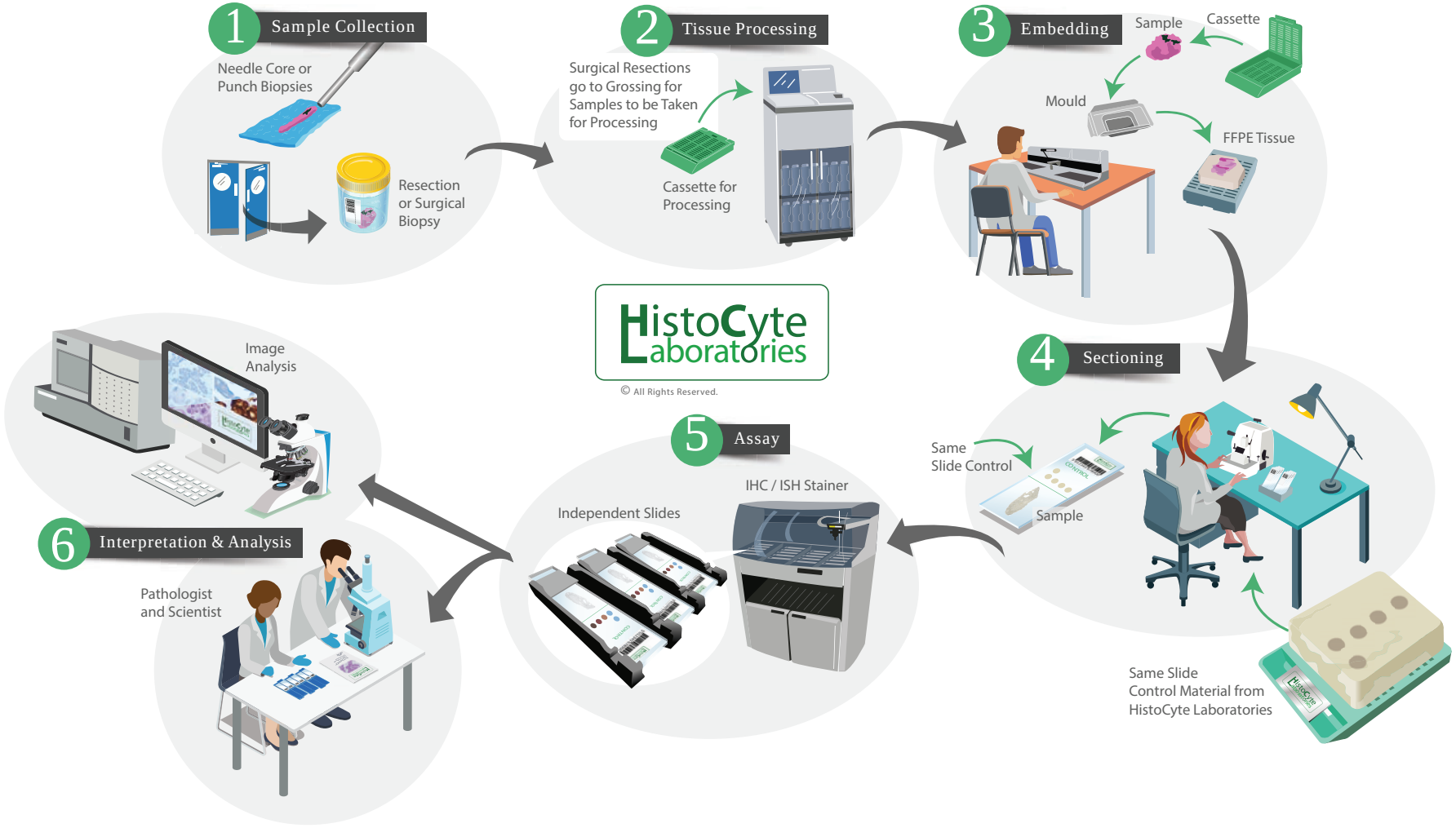
Quality in Control

medac

Cell Microarray Blocks
and Pre-cut Slides
for Laboratory Quality Control

# Reliable Standardised Control Material

# Workflow Through a Pathology Laboratory




## 1. Sample Collection

Samples are taken in a variety of ways but **the critical element that affects samples is the cold ischemic time.** This is the time a sample leaves a patient to the point it is put in fixative such as formalin. There are guidelines that recommend that the **ischemic time should be kept below 1 hr.** This can be critical in breast when looking at hormone receptors and Her2. The longer the cold ischemic time, the greater the degradation of the sample, leading to a loss of tissue integrity and biomarker viability. The result will not truly reflect the status of the patient's disease.

## 2. Tissue Processing

The time that tissue spends in fixative (fixation time) can also have a profound effect on the preservation of antigens. Prolonged or variable fixation will affect diagnostic test results. As do different fixatives or relative formulations of formalin. For a variety of reasons, some laboratories will fix for longer periods than others. This can be due to geography; where samples have to travel great distances. Or where surgery performed on a Friday may result in samples not processed until Monday. **Specimens taken on a Friday may end up being fixed for three times as long as samples taken through the week.**

There are a number of different tissue processors employed in laboratories, each with its own nuances, leading to a lack of standardization. Each system manages the reagents (alcohol and xylene) differently for dehydration and clearing. This in turn can impact on reagent effectiveness. **Each laboratory believes they employ the right fixation procedures and tissue processing protocols.** Processing directly affects immunoreactivity, morphology and DNA/RNA viability. **Over fixed tissue masks the genuine biomarker expression** in a sample. **Reference laboratories receiving samples from a number of different centers cannot possibly compensate for the different fixatives, fixation time and processing protocols used for each sample they receive.**

## 3. Embedding

**By the time a sample is embedded any effect on tissue integrity or the biomarkers will have already happened, most irreversibly.** The sample will be orientated in a mold and embedded in paraffin wax. The wax will not have an effect on the viability of the sample or the biomarkers (unless it is not removed efficiently during the assay) but it can have an effect on the cutting of the block.

## 4. Sectioning

Good quality wax will ensure the block is relatively easy to cut. Sections should be cut at 3µm but many will state 3-5µm. Blocks are cooled on ice or freezer blocks, hardening the wax for cutting and shrinking it. As the block warms it expands. Therefore, as a block is being cut, the first sections will be thinner than the last in any one round of cutting.

Typically these do not affect the immunohistochemistry (IHC) results. However, **in some instances a thicker section can make some results look more positive.** This is most important in assays that are semi-quantitative, such as Her2. At this point, control material is added to the same slide. **Same slide controls are essential to demonstrate that the assay has performed appropriately.**

**Using internal control tissue will not allow one to control against any pre-analytical drift** such as fixation times or reagent effectiveness. If the controls and the samples are treated in the same manner, the laboratory will never know there is an issue. **Standardized external samples characterized and validated with standardized assays guard against the multiple sources of variation affecting samples, providing the best means to determine assay performance.**

## 5. Assay

Previously, slides being tested for the same biomarker were grouped together and tested together (batched) with a batch control. However, over the last 10-15 years the field has moved to automated platforms that treat slides independently. These more modern, flexible instruments require the use of same slide controls.

**Without a same slide control it is difficult to determine false negatives.** Additionally, with semi-quantitative assays, controls that demonstrate varying levels of expression are required to determine if it is working with the appropriate sensitivity. The requirement for same slide controls on tests also adds to the pressure on the laboratory to source and characterize sufficient quantities of control material. This has led to some laboratories still relying on the use of inappropriate batch controls.

**Without appropriate controls it is impossible to determine the sensitivity of the assay and appropriateness of the result.** By employing HistoCyte Laboratories Ltd products, laboratories can apply the appropriate control for their tests, demonstrating assay sensitivity with a cost-effective solution. Quality control is crucial for the laboratories to guard against inaccuracies in their results. **While upstream issues such as the pre-analytical variables can be hard to manage or prevent, having the right quality control material to demonstrate the assay has performed properly becomes paramount.**

## 6. Interpretation and Analysis

Scientists, pathologists and image analysis platforms can only assess what is in front of them. In checking the quality of the assay, the scientist need only see that the control has worked effectively. **Pathologists will look for internal controls within the sample.**

**When there is a lack of internal controls they rely on the quality control sample.** If this is a batch control, it may bear no relation to how the original case was performed by virtue of the slides being tested independently. A batch control will therefore not guarantee to demonstrate how all the other slides have performed.

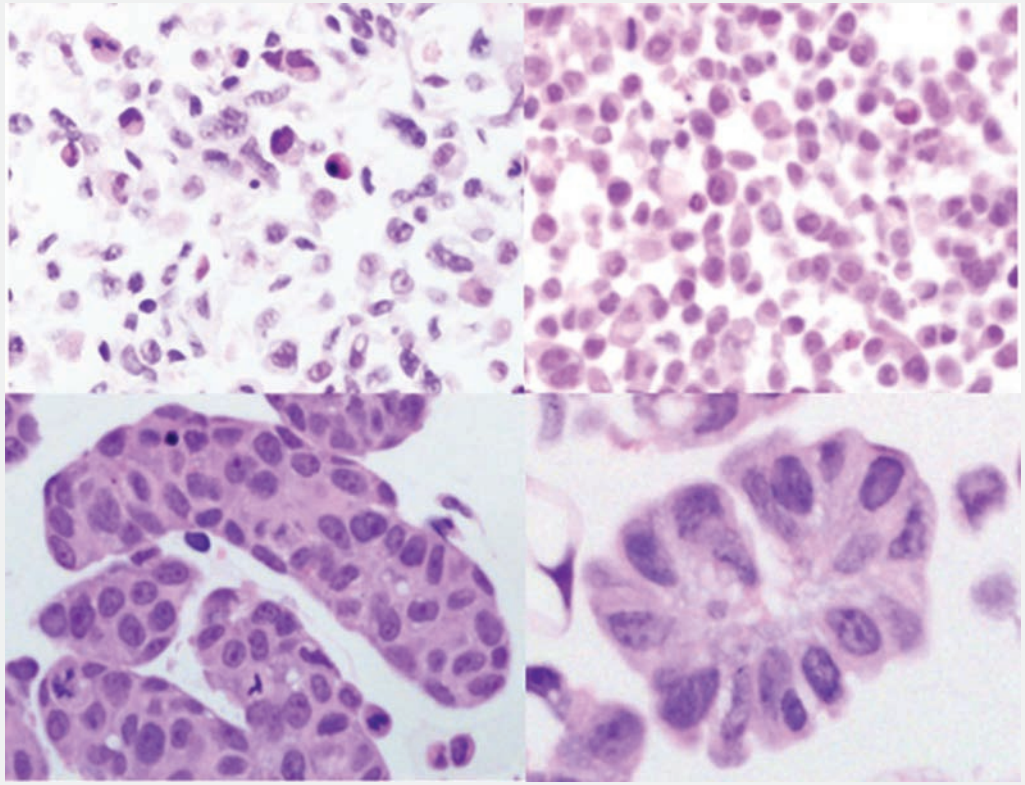
**This is where same slide controls are imperative.** With standardized, characterized cell lines from HistoCyte Laboratories Ltd, the scientists quickly become familiar with their performance and can easily tell if the assay has under or over performed. Additionally, the pathologist need only look up the slide to view the control rather than chase the batch control slide that may be elsewhere. For image analysis, while many systems have sophisticated means of calibrating themselves, they can only analyse what is on the slide. If they are calibrated to known standardized material such as the HistoCyte Laboratories Ltd analyte controls, the system can establish whether the assay was performed appropriately. It can determine how much more strongly or weakly an assay is performing.

# Quality in Control

**Established in 2014, HistoCyte Laboratories Ltd was founded by colleagues with over 30 years of experience in the development of diagnostic products, including cell line controls for use in diagnostic assays.**

**HistoCyte Laboratories Ltd** recognises that the identification of suitable archive tissue for use as controls in slide based assays can be difficult due to heterogeneous expression of biomarkers, fixation artefacts, or rarity of their expression in the tissue samples available.

Additionally, as laboratories move to ISO: 15189 accreditation, there is an increased requirement for the use of independent third party control material. Both the pressures to find rare control tissue types and the need for large quantities of control material can be alleviated through the use of products from **HistoCyte Laboratories Ltd.**



## What Makes us Different?

**The novel technology developed by HistoCyte Laboratories Ltd allows us to manufacture quality high density cell blocks composed of any cell line. These are ideal for quality control in any laboratory.**

Through careful selection and screening of cell types we can generate a range of positive and negative controls to determine effective performance of reagents used in slide based assessments.

Using cells originating from tumours means we can observe the expression of a variety of biomarkers with their associated pathologies. We can observe co-expression of biomarkers such as **HER2**, **ER** and **PR** in our Breast Analyte Control.

Importantly we understand the need for quality alongside the cost constraints of the pathology laboratory.

We therefore have developed cell microarray blocks that are cost effective for laboratories to use as same slide controls.
Thus enabling them to meet the requirements of ISO:15189.

**We like to think we have Quality in Control.**

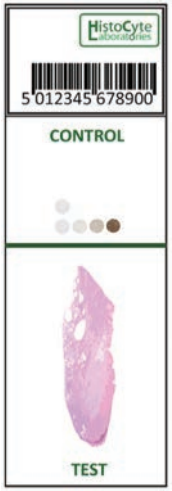
# Breast Analyte Control[DR]

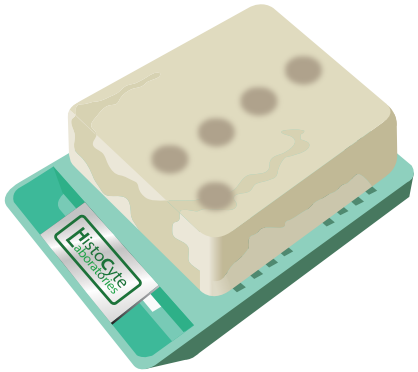
**Breast Analyte Control[DR] is part of the *Dynamic Range* of HistoCyte Products.**

When a Dynamic Range or a control refelecting the sensitivity of an assay is required then the **Breast Analyte Control[DR]** is ideal. This product contains four cells of varying expression for a variety of breast biomarkers, including Her2, Estrogen Receptor and Progesterone Receptor. It also includes an osteosarcoma negative control.

**Breast Analyte Control[DR]** is available as pre-cut slides (2 slide and 5 slide) and cell microarray blocks.

| Format | Product Code |
|---|---|
| 2 Slide | HCL016 |
| 5 Slide | HCL017 |
| Block | HCL018 |



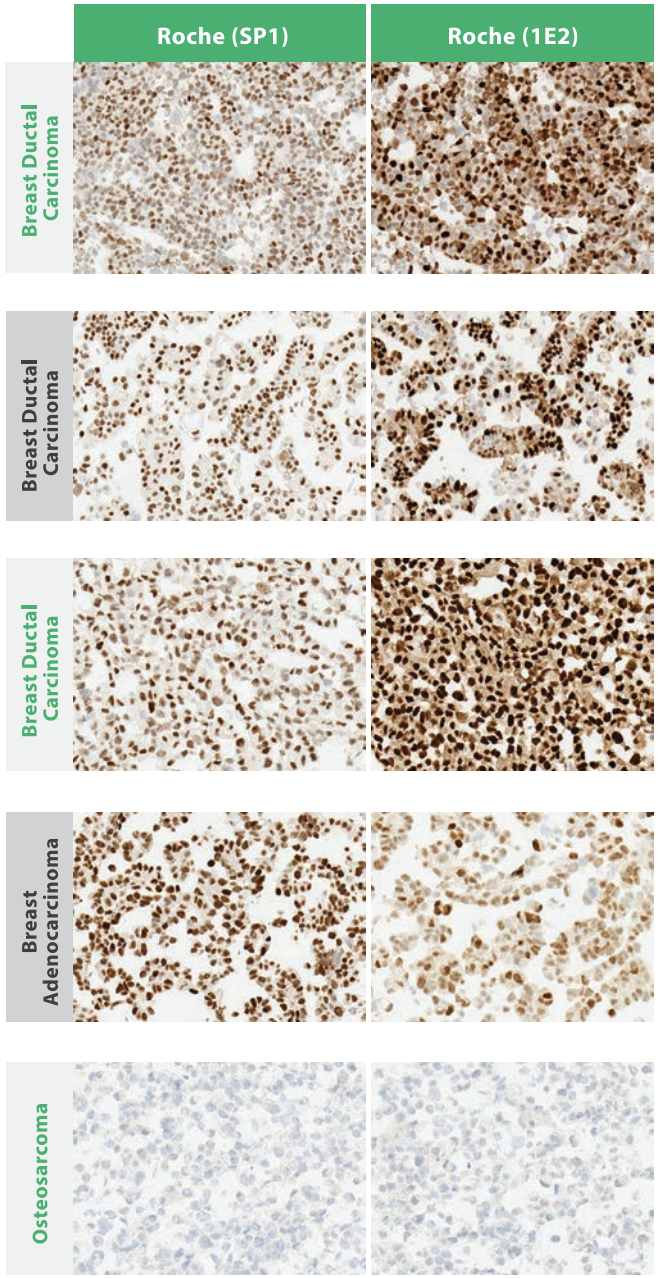

## QC Scoring: ER/PR

**The heterogeneous cells are the key to determining how significantly the assay performance fluctuates.**
If the assay over stains or under stains, more or less of the cells in the heterogeneous cores will stain. For both ER and PR there are three cores with heterogeneous expression.

Due to the heterogeneous expression in the cores Quickscore or Allred scoring is not useful in assessing the cells. This is specifically for clinical assessment in tissue.

As the heterogeneous cell cores provide the utility for the control, a QC scoring method needs to be applied rather than a clinical one.
**The table below has an example of a scoring method that easily conveys assay performance to the pathologist.**

| QC Score | Result | Definition |
|---|---|---|
| 1 | Query/fail | Control not staining as normal. Too weak. Repeat if necessary upon review of test sample. |
| 2 | Pass | Performance lower than expected but within tolerance |
| 3 | Pass | Performance as expected |
| 4 | Pass | Performance higher than expected but within tolerance Control not staining as normal. |
| 5 | Query/fail | Too strong. Repeat if necessary upon review of test sample. |



# Her2 1+ versus 2+ Cells

**Her2 2+ is difficult to obtain in laboratories and not always consistent.**
Tumour is often highly heterogeneous either because of the way the protein is expressed or because of the affects of fixation and processing.
2+ creates the greatest degree of inter-observer discordance[1].

**"Is it a weak 2+ or a strong 1+?"**


There is no linear correlation between 0/1+/2+ and 3+ (orange vs blue lines).
The linear relationship between 0, 1+ and 2+, makes 1+ cell line is the best predictor of assay drift (red line).

**There are two 1+ cells in the Breast Analyte Control[DR] allowing a laboratory to demonstrate consistency within the control.**

1. Turashvili G, Leung S, Turbin D, Montgomery K, Gilks B, West R, Carrier M, Huntsman D, Aparicio S. Inter-observer reproducibility of Her 2 immunohistochemical assessment and concordance with fluorescent in situ hybridization (FISH): pathologist assessment compared to quantitative image analysis. BMC Cancer. 2009 May 29;9:165
2. Receptor load figures taken from Bond Oracle HER2 IHC System Interpretation Guide, Leica BioSystems, 2011.



# Her2 QC Scoring

**Diagnostic algorithms are not necessarily a means to assess Quality.**

While Her2 are scored 0, 1+, 2+, 3+ controls in laboratories are often scored using an adapted system to convey how the assay has performed. See table below.

| | QC Score | | | | | | |
|---|---|---|---|---|---|---|---|
| Her2 | 0 | 0/1+ | 1+ | 1/2+ | 2+ | 2/3+ | 3+ |

This "grey" area is necessary to reflect the tolerance of the assay and impart to the pathologist how well the assay has performed.

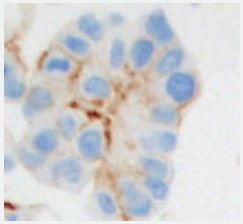
This QC score can be considred by the pathologist assessing the case.

# What Not to Score

**Some elements in the 1+ Cell can stain strongly like the luminal brush.**

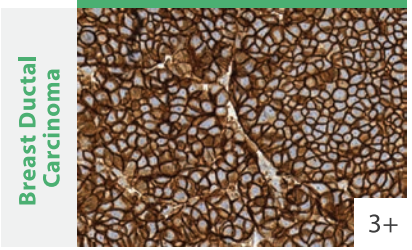
Seen in tissue samples, it too should be ignored and not considered in the scoring of the cells.

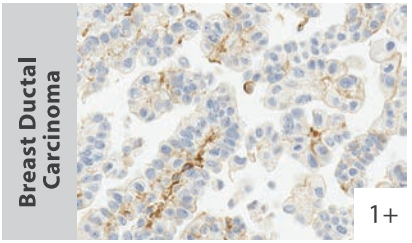
These are ideal for quality control us in any laboratory.

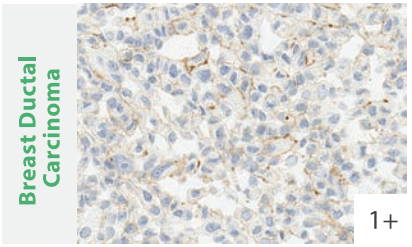


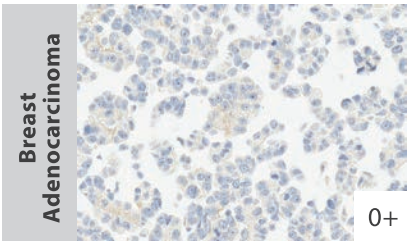
**Luminal brush border**

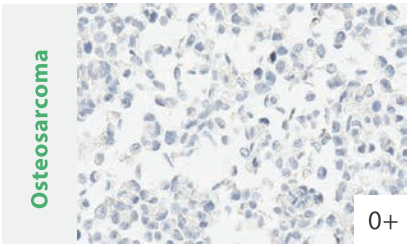
**Roche (4B5)**



# ALK-Lung and ALK-Lymphoma

**Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor encoded by the ALK gene.**

The ALK gene is found on the short arm of chromosome 2. As an oncogene it was first identified as a translocation in anaplastic large cell lymphoma (ALCL) t(2;5)(p23;q35).

In this instance the translocation caused a fusion product with the nucleophosmin gene: NPM-ALK.[2]

In reality the ALK translocation is a promiscuous event and associated with numerous fusions in multiple malignancies.[1]

## Detecting ALK

There are many assays for detecting ALK including PCR and FISH. However, the most commonly used antibodies on the market include clones 5A4, D5F3 and ALK1 .[2]
These all recognise the C-terminus (see respective vendor data sheets), the green sections in Figure 1, below. This is the conserved ALK region harbouring the tyrosine kinase domain.

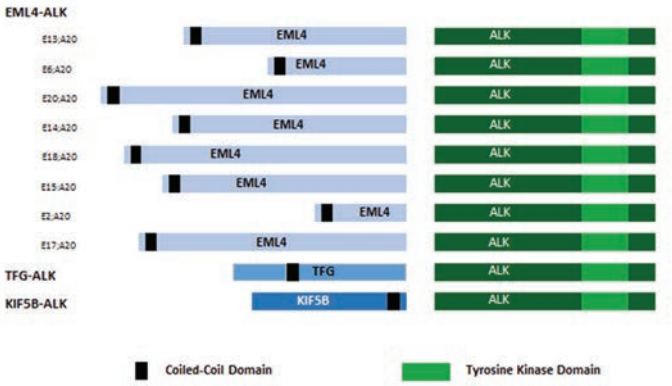


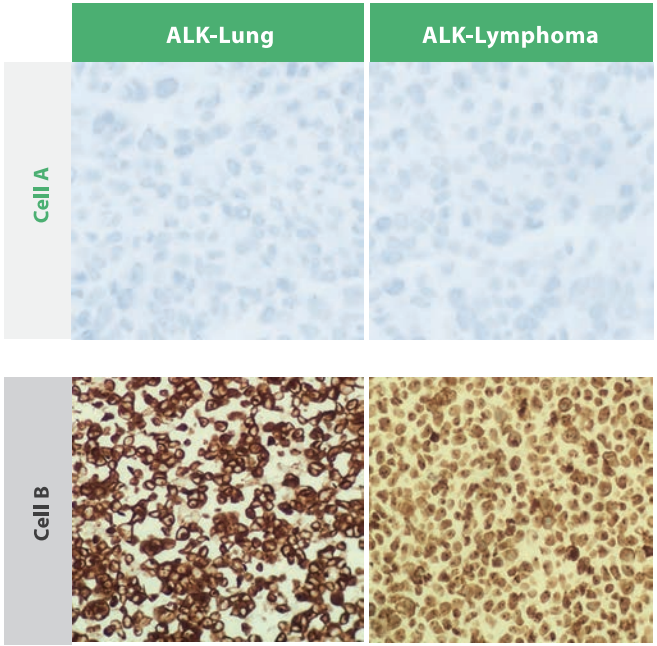

Figure 1. ALK translocations (adapted from reference 3)

Therefore, all of these antibodies should recognise the ALK fusion proteins. In practice the efficacy of the antibody or its affinity for the target epitope, the relative availability of fusion protein and appropriate epitope retrieval and IHC protocols mean that variation is seen from laboratory to laboratory. This is evidenced in external quality assurance programs.[2]

## The Right Concentration

Due to the different expression levels of these fusion proteins the antibody, while recognising the conserved ALK tyrosine kinase domain, needs to be optimised appropriately in each disease. For example using an ALK antibody optimised to NPM-ALK for use in anaplastic large cell lymphoma, will not be sensitive enough to adequately detect the spectrum of expression of the EML4-ALK fusion protein in non-small cell lung carcinoma.
For this reason **HistoCyte Laboratories Ltd** have developed two specific ALK controls for both lung (EML4-ALK) and lymphoma (NPM-ALK). Each containing a positive and negative cell core. Available as cut slides and cell microarray blocks. Suitable for IHC and ISH.


| Type | Format | Product Code |
|---|---|---|
| ALK-Lung | 2 Slide | HCL007 |
| | 5 Slide | HCL008 |
| | Block | HCL009 |
| ALK-Lymphoma | 2 Slide | HCL010 |
| | 5 Slide | HCL011 |
| | Block | HCL012 |





1. Iragavarapu C, Mustafa M, Akinleye A, Furqan M, Mittal V, Cang S, Liu D. Novel ALK inhibitors in clinical use and development. J Hematol Oncol. 2015 Feb 27;8(1):17
2. UKNEQAS Journal: Immunocytochemistry. Run 108/37. Assessments Dates: 5th-23rd January 2015.
3. Sasaki T, Rodig SJ, Chirieac LR, Jänne PA. The Biology and Treatment of EML4-ALK Non-Small Cell Lung Cancer. Eur J Cancer. 2010 July ; 46(10): 1773–1780.

# HPV/p16 Analyte Control Range

**Human Papillomavirus or HPV consist of more than 150 related viruses, over 40 of which can be sexually transmitted through direct skin-to-skin contact.**

These sexually transmitted HPV subtypes fall into two categories:

Low-Risk HPV, e.g. HPV 6 & 11, which do not cause cancer but are responsible for 90% of genital warts cases.

High-Risk or Oncogenic HPV which can cause cancer. At least 15 high-risk HPV subtypes have been identified. Two of these, HPV 16 & 18, are responsible for the majority of HPV-related cancers.[1, 2]

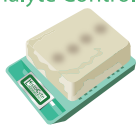
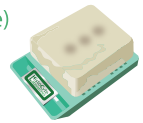
## HPV in Cancer

Virtually all cases of cervical cancer are caused by HPV infection, with HPV 16 & 18 detected in 70%.[1,2] HPV 16 is responsible for around 85% of anal cancers and HPV 16 & 18 account for approximately 50% of vaginal, vulval and penile cancers.[3] Within the last 20 years, the incidence of HPV-associated oropharyngeal cancer has increased, particularly among men. HPV 16 has been identified in around 50% of oropharyngeal cancers in the US.[4]

**It has been estimated that, by 2020, HPV will cause more oropharyngeal cancers than cervical cancers in the US.[5]**

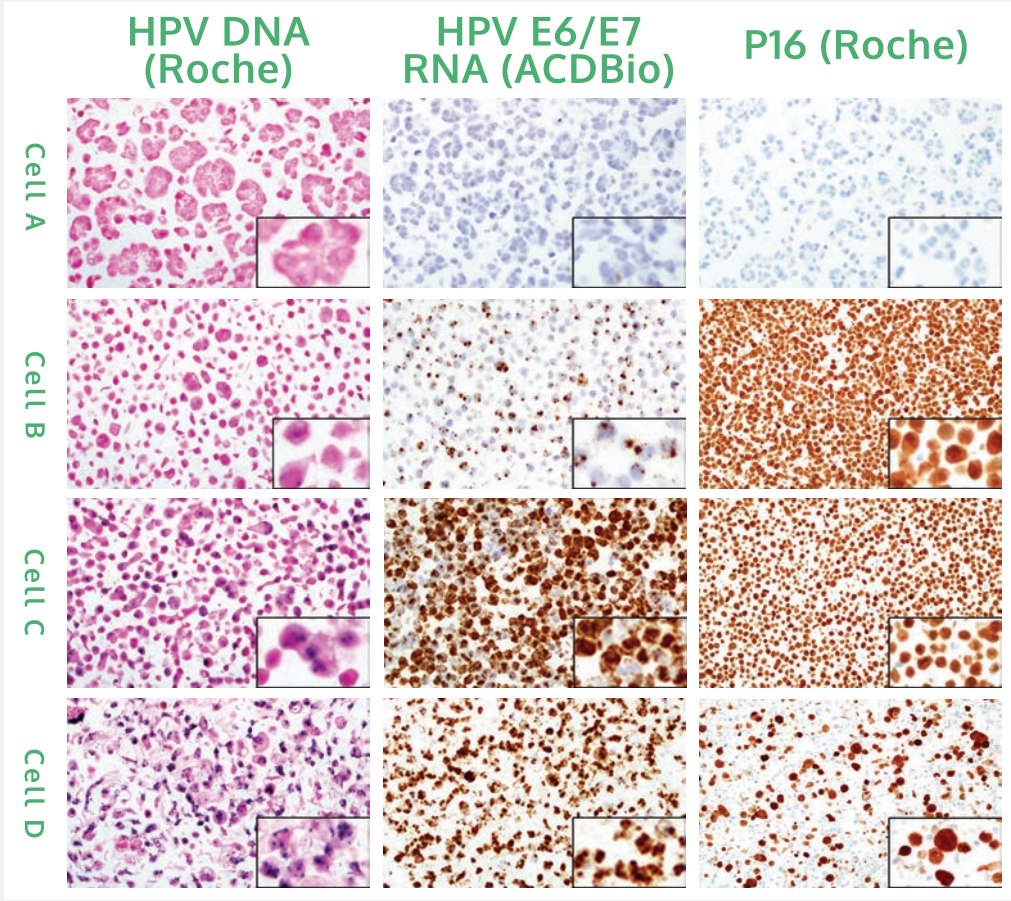
## Detecting HPV and p16

HPV infection is detected using assays that detect viral DNA or RNA within the cell. Additionally p16 is commonly used as a surrogate marker of oncogenic HPV infection and can be demonstrated using immunohistochemistry. HPV DNA is most commonly assessed by PCR and in-situ hybridisation (ISH). Recently, more sensitive ISH assays, able to detect HPV mRNA E6 & E7, have come into routine use.

| Type | Format | Product Code |
|---|---|---|
| HPV/p16 Analyte Control[DR] (Four core)  | 2 Slide | HCL001 |
| | 5 Slide | HCL002 |
| | Block | HCL003 |
| HPV/p16 Analyte Control (Three core)  | 2 Slide | HCL004 |
| | 5 Slide | HCL005 |
| | Block | HCL006 |


1. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007; 370(9590):890–907.
2. Muñoz N, Bosch FX, Castellsagué X, et al. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. International Journal of Cancer 2004; 111(2):278–285.
3. Watson M, Saraiya M, Ahmed F, et al. Using population-based cancer registry data to assess the burden of human papillomavirus-associated cancers in the United States: overview of methods. Cancer 2008; 113(10 Suppl):2841–2854.
4. Jayaprakash V, Reid M, Hatton E, et al. Human papillomavirus types 16 and 18 in epithelial dysplasia of oral cavity and oropharynx: a meta-analysis, 1985–2010. Oral Oncology 2011; 47(11):1048–1054.
5. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. Journal of Clinical Oncology 2011; 29 (32):4294–4301.


HistoCyte Laboratories Ltd have developed a four core product **HPV/p16 Analyte Control[DR]** and three core **HPV/p16 Analyte Control** (has no cell B).




| Cell Lines | HPV Gene Status | E6/E7 mRNA | p16 Expression |
|---|---|---|---|
| A: Human Breast Adenocarcinoma | Negative | Negative | Negative |
| B: Human Cervical Squamous Cell Carcinoma | Low (1-2) HPV gene copy | Low | High |
| C: Human Cervical Adenocarcinoma | Medium HPV gene copy | High | High |
| D: Human Epidermoid Carcinoma | High HPV gene copy | High | High (heterogeneous) |

# PD-L1 Analyte Control[DR]

Programmed death ligand 1 (PD-L1) is a 40kD type 1 transmembrane protein. Synonyms include:

- **CD274**
- **B7 homolog 1 (B7-H1)**

PD-L1 is a checkpoint regulator in immune cells[1], it is expressed on immune or non- hematopoietic cells[2]. Expression of the protein is seen during pregnancy where it has a role in supressing the immune system. PD-L1 induces an inhibitory signal in activated T-cells and promotes T-cell apoptosis[2].

PD-L1 has been observed to be over expressed in a number of different cancer types and is believed to be a potential means by which the cancer cells can evade the immune system. Overexpression of PD-L1 correlates with poor disease outcomes[3].

The expression of PD-L1 within cancer is not restricted to a single type of cancer and as such it has become a target for anti-cancer drug development. Currently there are a number of anti-PD-L1 clinical trials ongoing, focusing on the following tumour types:

- **Lung cancer**
- **Bladder cancer**
- **Kidney cancer**
- **Haematological cancer**
- **Breast cancer**
- **Colorectal cancer**
- **Melanoma**
- **Solid tumours**

The diagram below (Figure 1.) illustrates the interaction between the tumour cells and the immune system, whereby the anti-PD-L1 antibody blocks the ability of the ligand to bind with the PD-1 receptor. Thus preventing the inhibitory feedback that would otherwise be stimulated.

## Detecting PD-L1

A number of different methods are used to measure PD-L1 expression, these include:

Molecular methods such as real-time polymerase chain reaction using products such as TaqMan® gene expression assay from ThermoFisher.

Fluorescence in situ hybridisation (FISH) probes for the detection of PD-L1 DNA, Advanced Cell Diagnostics provide an RNAscope product for the detection of PD-L1 mRNA. A number of antibodies are available for the immunohistochemical detection of PD-L1, these include clones E1L3N (Cell Signalling Technology), SP263 (Roche), and 28-8 (Dako). These antibodies are available in a variety of different formats.

1. Iragavarapu C, Mustafa M, Akinleye A, Furqan M, Mittal V, Cang S, Liu D. Novel ALK inhibitors in clinical use and development. J Hematol Oncol. 2015 Feb 27;8(1):17
2. UKNEQAS Journal: Immunocytochemistry. Run 108/37. Assessments Dates: 5th-23rd January 2015.

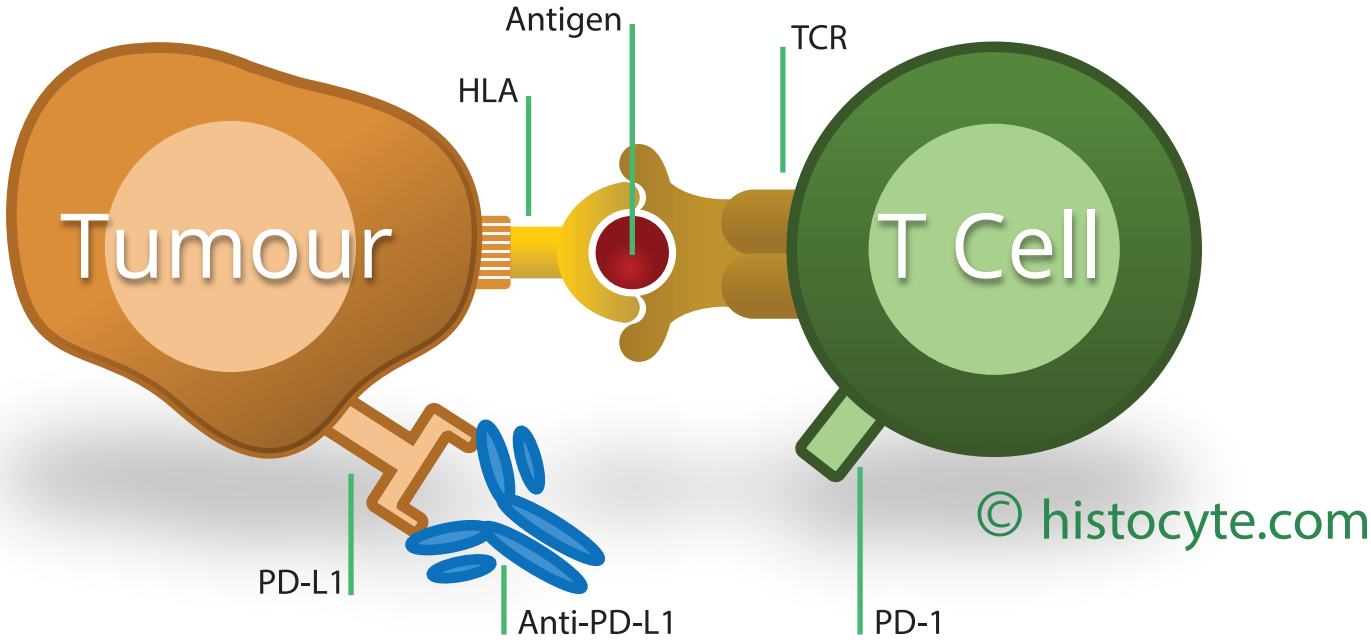


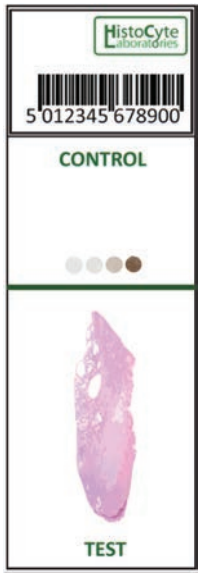

Figure 1. The human leukocyte antigen (HLA) on the tumour cell presents tumour protein which is detected through the T receptor (TCR). Upon recognising "tumour protein" the T cell initiates a cytotoxicity event, which would otherwis inhibited by the interaction between PD-L1 and PD-1 on the T cell.

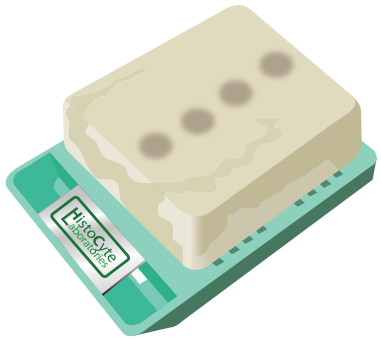
# HistoCyte Laboratories PD- L1 Analyte Control[DR]

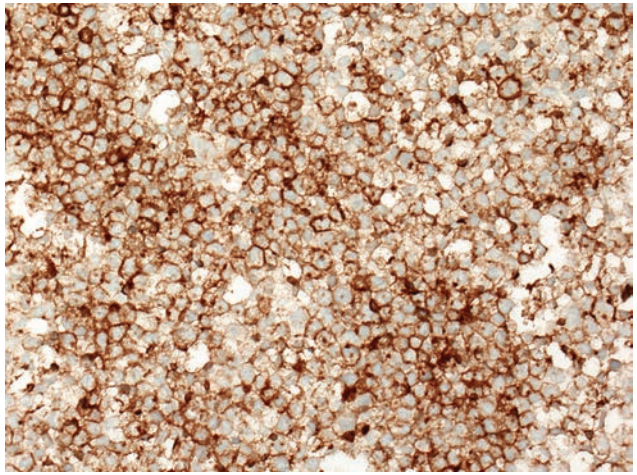
The product consists of four different cell lines with PD-L1 expression levels of high, medium, low and negative. The product was developed using the E1L3N PD-L1 antibody. However, it has also been independently tested at different sites using different PD-L1 antibodies with different protocols. In all cases the HistoCyte PD-L1 control provided the same high, medium, low and zero expression range.

Whilst the HistoCyte PD-L1 control product has not been validated with FISH assays, all HistoCyte products are designed to be suitable for FISH testing.

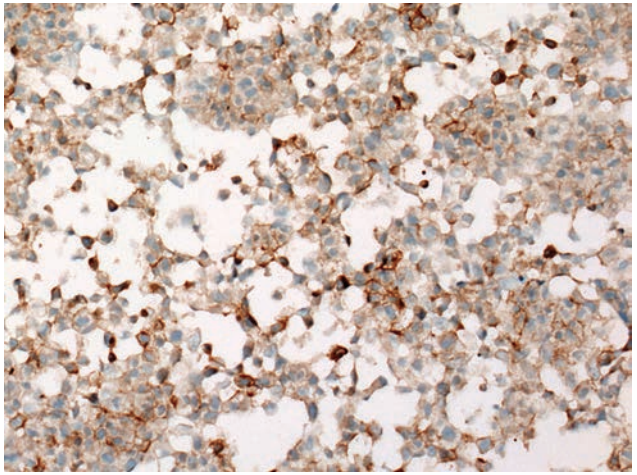
| Type | Format | Product Code |
|---|---|---|
| PD-L1 Analyte Control[DR] | 2 Slide | HCL019 |
| | 5 Slide | HCL020 |
| | Block | HCL021 |



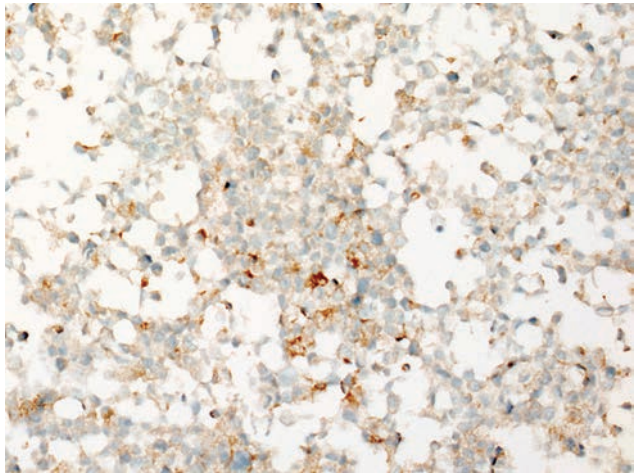





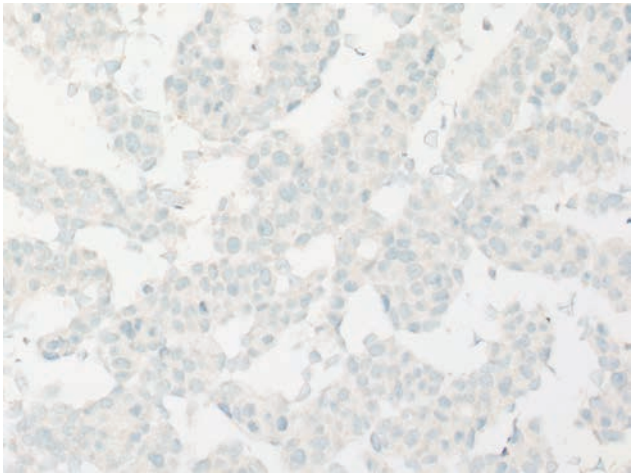
**High expression:** Strong staining in majority of cells.
Tumor Type: T cell non-Hodgkin Lymphoma



**Medium expression:** Convincing staining in majority of cells. Some strong staining. Tumor Type: Fibrosarcoma



**Low expression:** Faint staining in majority of cells. Occasional strong staining.
Tumor Type: Osteosarcoma



**Negative expression:** Absence of any genuine staining.
Tumor Type: Breast ductal carcinoma

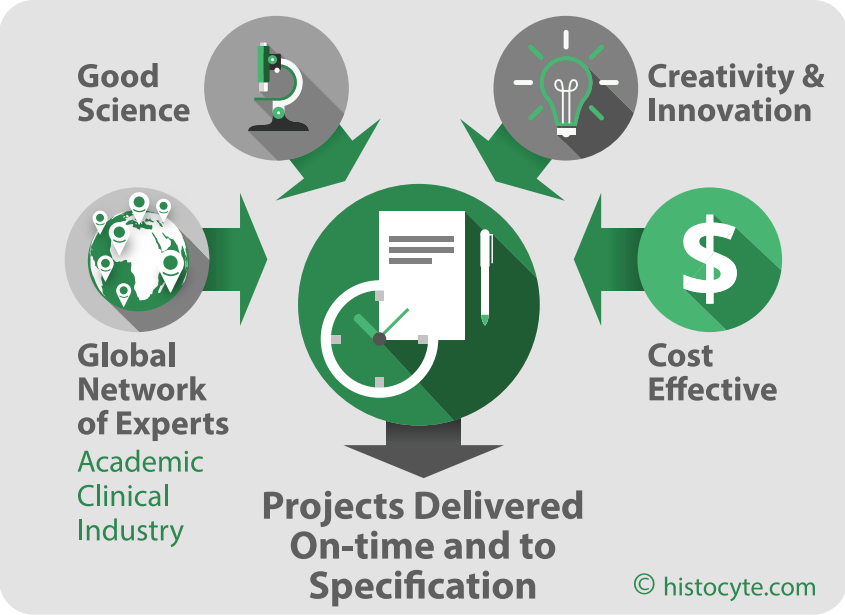
Staining performed on Roche Ventana Benchmark Ultra with Optiview and Cell Signalling Technologies RmAb Clone E1L3N.

# Contract Manufacture & Development

# Product Summary

**HistoCyte Laboratories Ltd can develop almost any combination of cells you require. We start with your needs and product requirements.**

1. Cross reference biomarker, tissue/pathology and available cells.
2. Freedom to operate search: license restrictions.
3. Obtain cell(s) +/- license.
4. Depending on cell line can have a development batch ready to assess in 4-8 weeks from receiving cells.




We can do the following:
1. Perform proof of concept studies.
2. Rapid scale up of batch sizes.
3. Assess across multiple manufacturers platforms at multiple sites.

Competitive and efficient contact us at:
**info@histocyte.com** for more information.

| Targets | Product Name | Format | Code |
|---|---|---|---|
| HPV/p16 | HPV/p16 Analyte Control[DR] (Four core with dynamic range of HPV gene copies) | Slide (2) | HCL001 |
| | | Slide (5) | HCL002 |
| | | Block | HCL003 |
| | HPV/p16 Analyte Control (Three core with standard range of HPV gene copies) | Slide (2) | HCL004 |
| | | Slide (5) | HCL005 |
| | | Block | HCL006 |
| ALK | ALK-Lung Analyte Control (Two core positive and negative for the EML4-ALK translocation) | Slide (2) | HCL007 |
| | | Slide (5) | HCL008 |
| | | Block | HCL009 |
| | ALK-Lymphoma Analyte Control (Two core positive and negative for the NPM-ALK translocation) | Slide (2) | HCL010 |
| | | Slide (5) | HCL011 |
| | | Block | HCL012 |
| Breast Analyte Control | Breast Analyte Control (Two cores, one positive for Her2, ER and PR. The other negative) | Slide (2) | HCL013 |
| | | Slide (5) | HCL014 |
| | | Block | HCL015 |
| | Breast Analyte Control[DR] (Five cores with a dynamic range of expression of Her2, ER and PR. Including negative control) | Slide (2) | HCL016 |
| | | Slide (5) | HCL017 |
| | | Block | HCL018 |
| PD-L1 | PD-L1 Analyte Control[DR] (4 cores with a dynamic range of PD-L1 expression) | Slide (2) | HCL019 |
| | | Slide (5) | HCL020 |
| | | Block | HCL021 |

HistoCyte Laboratories

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