HistoCyte Laboratories

Quality in Control

NTRK Analyte Controls

Product Introduction

Product Codes:

HCL038, HCL039, HCL040

Issue 1. 210609

Contents

Product Name	Format	Code
NTRK Analyte Control (Two cores one negative and one positive for TrkA protein)	Slide (2)	HCL038
	Slide (5)	HCL039
	Block	HCL040

(For research use only)

Quality Control

One of the requirements of quality standardization is the appropriate use of controls. These need to be robust enough for IHC and in situ hybridization (ISH), be reproducible and cost-effective. Additionally, the control material should be consistent from batch to batch and throughout the block it is cut from.

Same slide control versus batch controls

In laboratories with automated platforms these controls need to be on the same slide. Batch controls are typically not representative of how slides have been treated as the instruments treat the slides completely independently.

External Quality Assurance

External quality assurance (EQA) schemes or proficiency testing (PT) have shown standardized assays typically perform better than laboratory developed tests (LDTs). In 2017 over 60% of UKNEQAS participants in RUN118/47 were using standardized ER vendor assays. Again in the NordiQC assessment B25 in 2018 >80% were using standardized ER assays.

Cell Lines as Controls

The issue with tissue

Laboratories often struggle for low and intermediate expressing material that is consistent, one example being HER2 2+ tissue. Not only is it hard to find tissue in sufficient amounts, but biomarker expression can also vary throughout tissue, often due to a number of factors including but not limited to:

- Fixation
- Processing artefact
- Heterogeneity of the protein, see Figure 1 (taken from Nitta H *et al*[1])

This means that tissue selected for use as control can vary to the point that it makes its use as a control redundant.

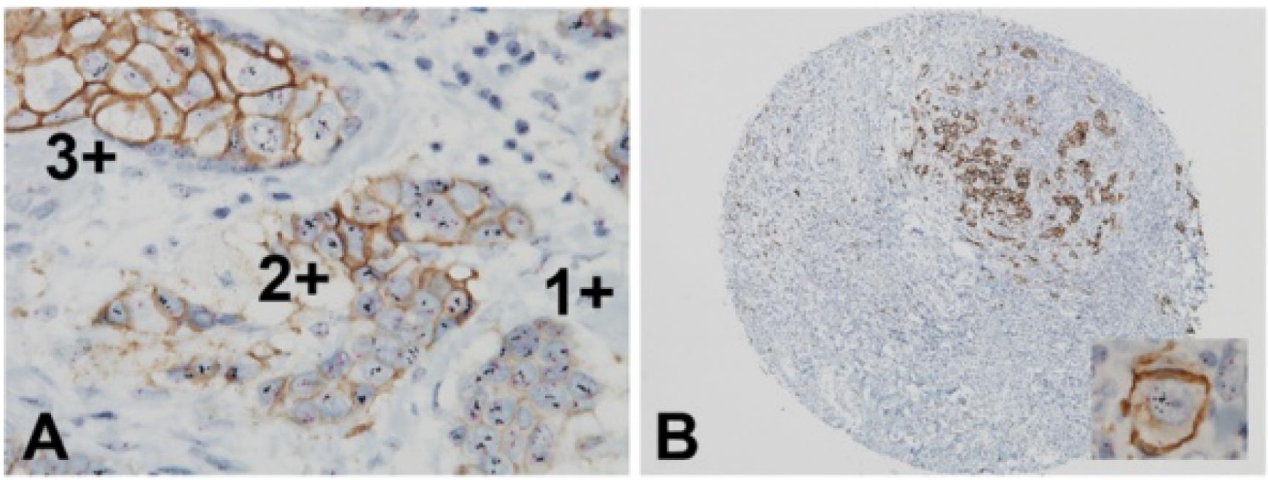


Figure 1. Results of HER2 gene-protein staining of FFPE breast cancer tissues exhibiting heterogeneity of HER2 positive tumor cell populations or isolated tumour cell populations. (A) The HER2 gene-protein assay demonstrated the heterogeneity of HER2 positive tumour cell populations in FFPE breast cancer tissues. In the sample shown, cell populations with HER2 IHC scores of 3+, 2+ and 1+ neighbor each other and all tumor populations present amplified *HER2* gene. However, the HER2 IHC 3+ tumor cell population contains dispersed *HER2* gene copies while the HER2 IHC 2+ and 1+ population contains clustered *HER2* gene copies [40x]. **(B)** The HER2 gene-protein assay clearly visualized small groups of HER2 3+IHC breast cancer cells [4x]. The insert shows an isolated individual HER2 IHC positive tumor cell with *HER2* gene amplification [100x].[6]

1. Nitta et al. Diagnostic Pathology 2012, 7:60

Cell lines

Cell lines are typically included in or with assays as pre-cut slides. These are not designed for use as same slide controls and pre-cut slides are not always practical for day to day use in a high volume laboratory. They are used by EQA schemes as standardized materials for their assessments. So while adequately performing by IHC or FISH, the preparations are often sparse and the cellular integrity or morphology is generally poor. So while they can be reproducibly manufactured to provide standardized material there is room for improvement.

Our solution

HistoCyte Laboratories provide cell lines that are compact and typically "tissue-like". In particular the breast ductal carcinoma cells often create "pseudo-acini" producing a more tissue like appearance. The morphology of our cells means that they can tell you more about how the slide has been treated. It is quite obvious when the morphology is disrupted. The HistoCyte Laboratories cell lines are intended to be used for quality control only. They are standardized, developed and manufactured to provide consistent results throughout the block. This is what differentiates them from tissue controls. It should be remembered that these still need validating in each laboratory that adopts them.

Tissue is still important

It is important to remember that HistoCyte controls are a quality control material designed only to demonstrate that the assay has worked consistently. They reduce the burden on a laboratory to identify and obtain suitable materials for use as a same slide control. This means tissue can be preserved for other uses such as trouble shooting and validations.

What is NTRK?

NTRK is a group of three Neurotrophic Tyrosine receptor Kinase genes: NTRK1, NTRK2 and NTRK3, each encode for the respective proteins TrkA, B and C. The external domain of the Trk receptors binds neurotrophin causing receptor dimerization, transphosphorylation and thus down stream signalling.

The Role of NTRK in Cancer

These NTRK genes are readily spliced, the resulting proteins end up with the C-terminal containing the tyrosine kinase/signalling part of the Trk protein. These fusion proteins are constitutively activated and drive tumorigenesis.

The first NTRK fusions were described in colorectal carcinomas[1]. However, since then they have been described in a wide variety of tumours including soft tissue tumours (pediatric and adult), breast, salivary gland, lung, colon and thyroid[2].

1. Nature. 1986;319:743–8
2. Arch Pathol Lab Med. doi: 10.5858/arpa.2020-0400-RA

NTRK Assessment

There are a number of means to assess NTRK gene translocations, fluorescence in situ hybridization (FISH), reverse transcriptase-polymerase chain reaction (RT-PCR) and Next Generation Sequencing (NGS). Immunohistochemistry (IHC) is the most effective means of assessing protein. The most widely used is the EPR17341 clone. This is a Pan-TRK monoclonal antibody recognising the C-terminal domain of the Trk-A, Trk-B and Trk-C proteins.

There are pros and cons to each method. Typically formalin fixed paraffin embedded tissues are assessed by IHC and reflexed to a molecular method.

NTRK Analyte Control

The product consists of two cell lines: one positive for Trk-A WT protein (not a fusion protein). The second has no translocation or expression of any Trk proteins. NTRK Analyte Control is sold in two formats: pre-prepared slides (Figure 2) or as a cell microarray (CMA) paraffin wax block (Figure 3).

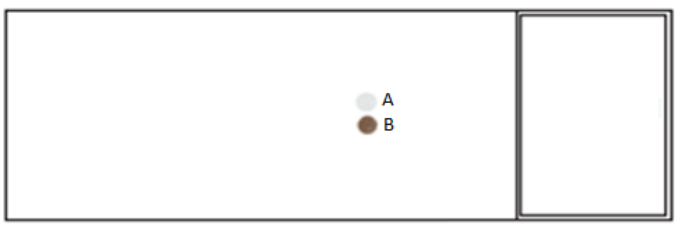


Figure 2: Cell Line Control Slide

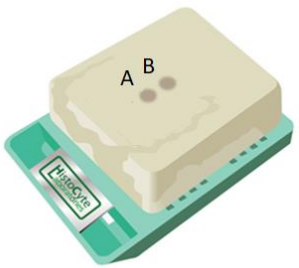
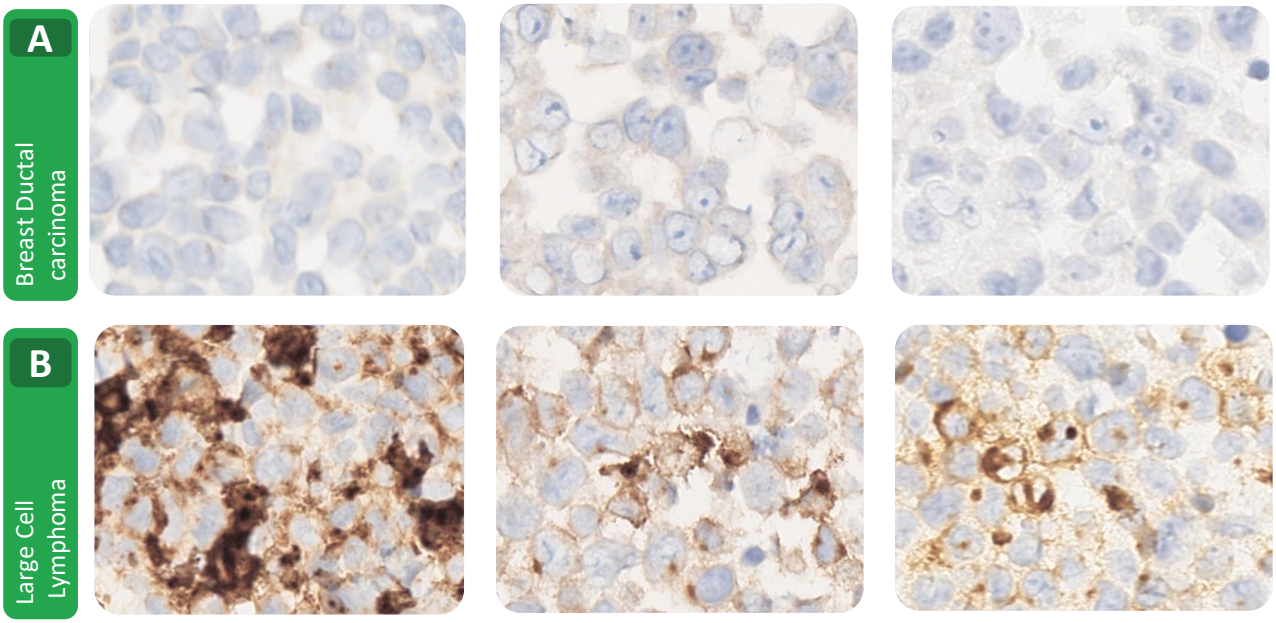


Figure 3: CMA block

The expression patterns of the 2 cell lines for Pan-Trk IHC are summarised below in the table.

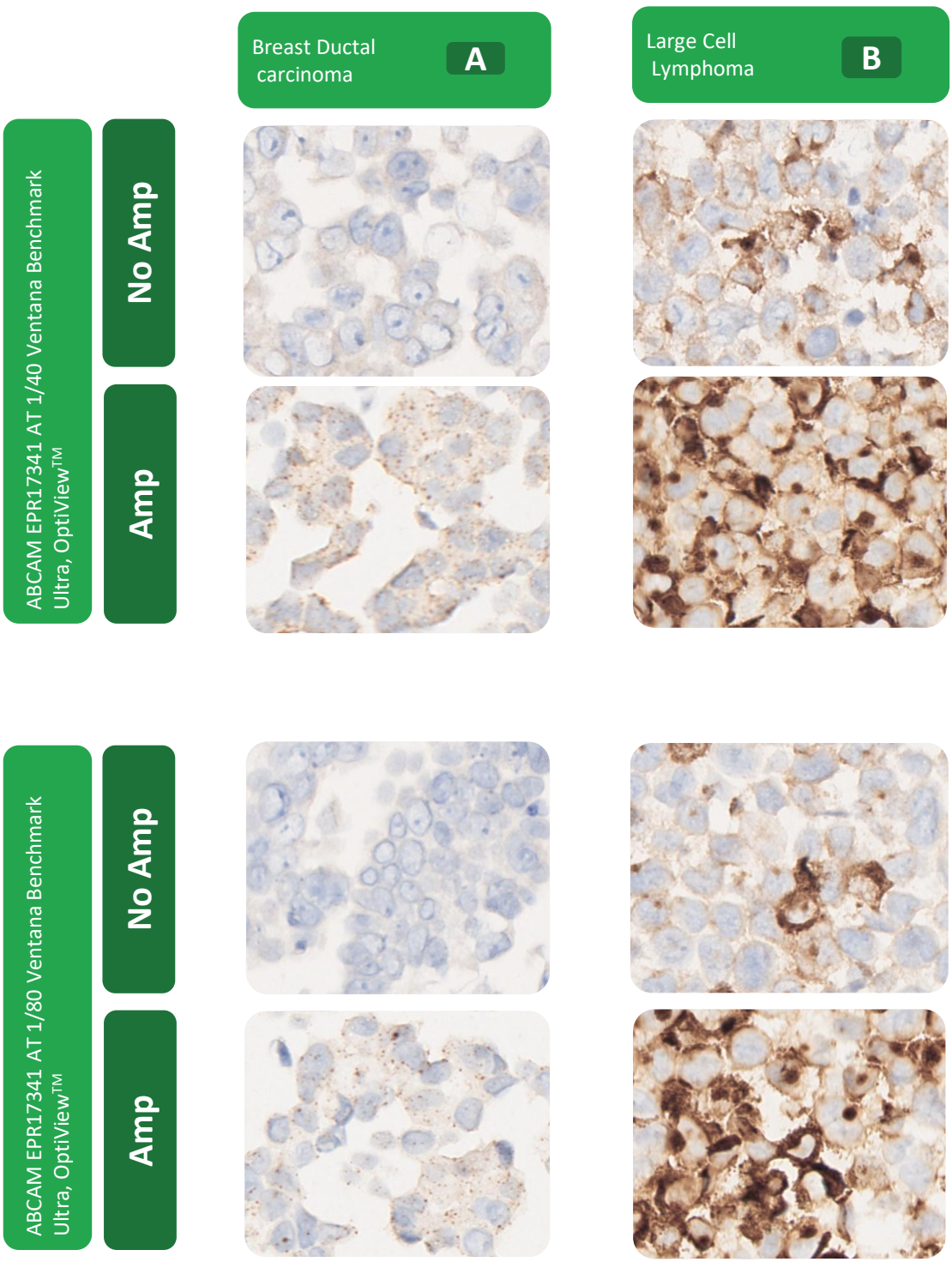
	Cell Lines	NTRK IHC
A	Breast Adenocarcinoma	Negative
B	Large Cell Lymphoma	Positive (WT Trk-A)

NTRK Analyte Control - IHC



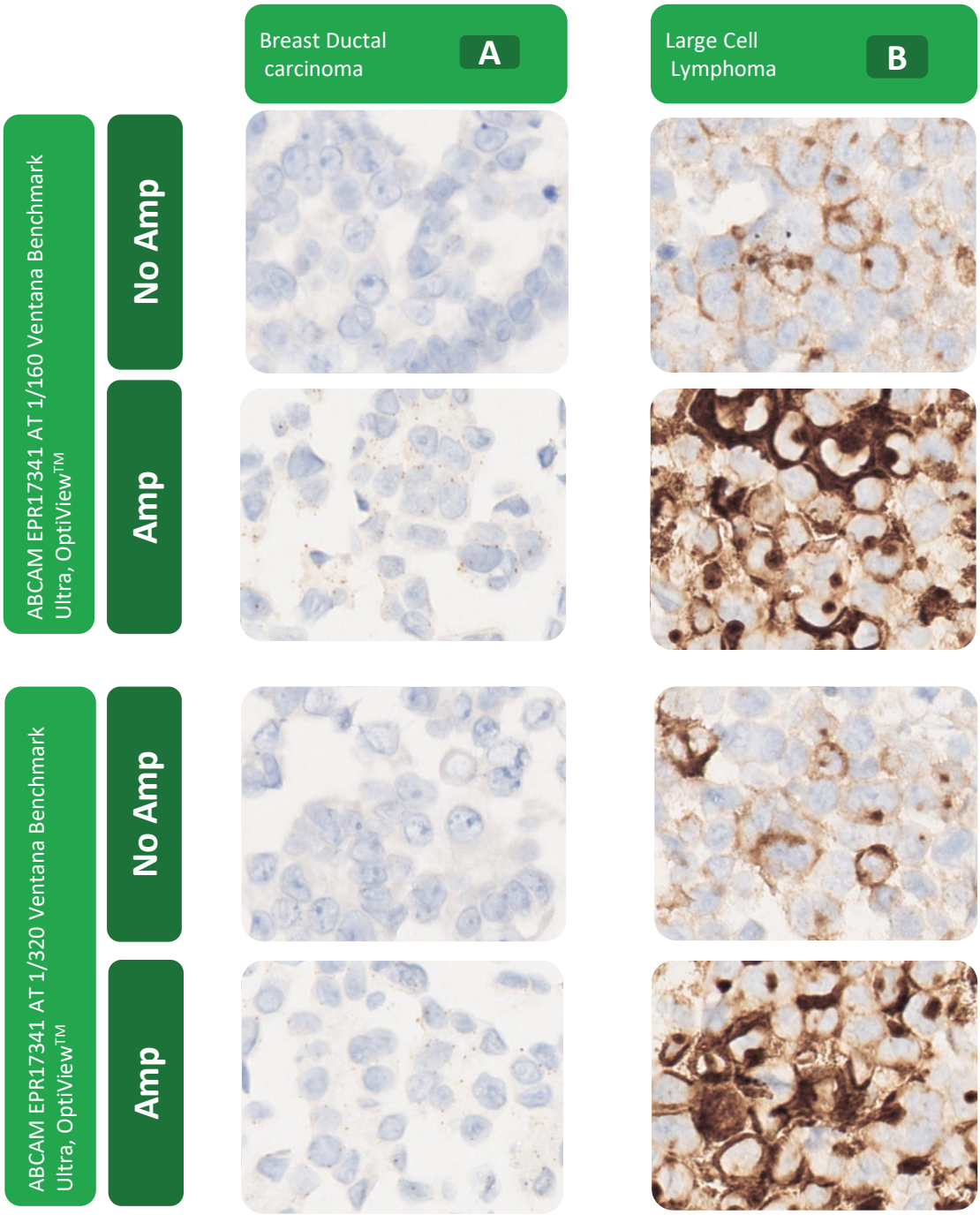
EPR17341 (AMP)	EPR17341 (No AMP)	EPR17341
ABCAM EPR17341 clone on the Ventana BenchMark ULTRA with VENTANA OptView. (Dil. 1/500) With Amplification	ABCAM EPR17341 clone on the Ventana BenchMark ULTRA with VENTANA OptView. (Dil. 1/40) With no Amplification	Ventana Roche pan-TRK (EPR17341) assay 790-7026 (on the Ventana BenchMark ULTRA. Standard protocol.

EPR17341 Titration



Images taken at x40 Hamamatsu NanoZoomer.

EPR17341 Titration



Staining in is membranous with frequent intense nucleoli staining. The NRG/TrkA is involved with the survival and pathogenesis of many non-neural malignancies including lymphomas[1].

1. Molecular Oncology 11 (2017) 1189–1207

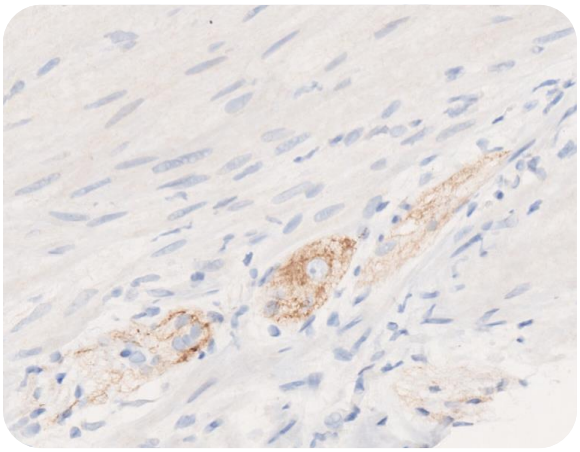
Images taken at x40 Hamamatsu NanoZoomer.

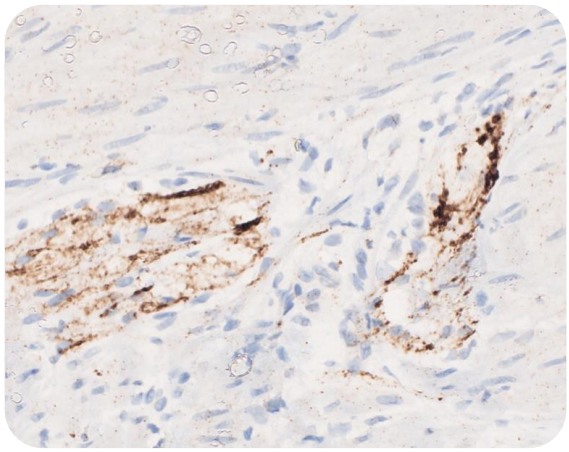
EPR17341 Titration

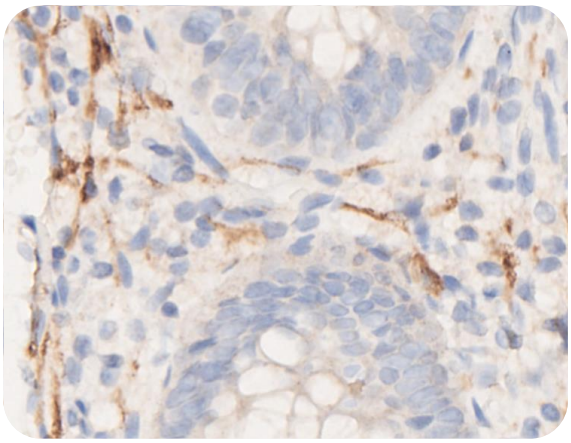
ABCAM EPR17341 AT Ventana Benchmark Ultra, OptiView™

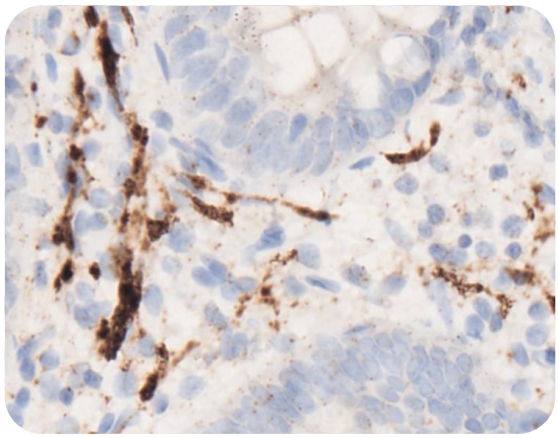
Appendix 1/40 no Amplification

Appendix 1/300 Amplification









Associated dilutions in appendix.

Clear staining of the ganglia in appendix. NTRK is also positive in neurones within the crypts.

Also Available From HistoCyte Laboratories Ltd

Product Name	Format	Code
HPV/p16 Analyte Control[DR] (Four cores: negative and three positive with dynamic range of HPV gene copies and p16 expression)	Slide(2)	HCL001
	Slide(5)	HCL002
	Block	HCL003
HPV/p16 Analyte Control (Three cores: negative and two positive for p16 and HPV gene copies)	Slide(2)	HCL004
	Slide(5)	HCL005
	Block	HCL006
ALK-Lung Analyte Control (Two cores: negative and a positive for the EML4-ALK translocation)	Slide(2)	HCL007
	Slide(5)	HCL008
	Block	HCL009
ALK-Lymphoma Analyte Control (Two cores: negative and a positive for the NPM-ALK translocation)	Slide(2)	HCL010
	Slide(5)	HCL011
	Block	HCL012
Breast Analyte Control (Two cores: negative and positive for HER2, ER and PR)	Slide(2)	HCL013
	Slide(5)	HCL014
	Block	HCL015
Breast Analyte Control[DR] (Five cores: variable levels of expression of HER2, ER and PR. Including negative control)	Slide(2)	HCL016
	Slide(5)	HCL017
	Block	HCL018
PD-L1 Analyte Control[DR] (Four cores: negative, low, intermediate and high levels of expression of PD-L1)	Slide(2)	HCL019
	Slide(5)	HCL020
	Block	HCL021
ROS1 Analyte Control (Two cores: negative and positive for ROS1 translocation SLC34A2-ROS1 and high expression of fusion protein)	Slide(2)	HCL022
	Slide(5)	HCL023
	Block	HCL024
ROS1 Analyte Control[DR] (Three cores: negative, FIG-ROS1 (very low fusion protein), SLC34A2-ROS1 (high fusion protein)	Slide(2)	HCL035
	Slide(5)	HCL036
	Block	HCL037
Sienna Cancer Diagnostics hTERT assay. 1ml of anti-hTERT mouse mAb.*	1ml	HCL025
HER2 Analyte Control[DR] (Four cores: 0, 1+ (both non-amplified), 2+ (equivocal) and 3+ (amplified))	Slide(2)	HCL026
	Slide(5)	HCL027
	Block	HCL028
Estrogen Receptor Analyte Control[DR] (Four cores: negative, low, intermediate and high)	Slide(2)	HCL029
	Slide(5)	HCL030
	Block	HCL031
Progesterone Receptor Analyte Control[DR] (Four cores: negative, low, intermediate and high)	Slide(2)	HCL032
	Slide(5)	HCL033
	Block	HCL034
NTRK Analyte Control (Two cores: negative and positive for WT TrkA protein)	Slide(2)	HCL038
	Slide(5)	HCL039
	Block	HCL040

HistoCyte Laboratories
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For more information email: **info@histocyte.com**
For orders email: **sales@histocyte.com**
Telephone: +44 (0) 191 603 1007
For your local distributor please visit **www.HistoCyte.com**