HistoCyte Laboratories

Quality in Control

ROS1 Analyte Controls

Product Introduction

Product Codes:

HCL022, HCL023, HCL024

HCL035, HCL036, HCL037

Issue 2. 200929

Contents

Product Name	Format	Code
ROS1 Analyte Control (Two cores one positive and one negative for ROS1 translocation)	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

(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.

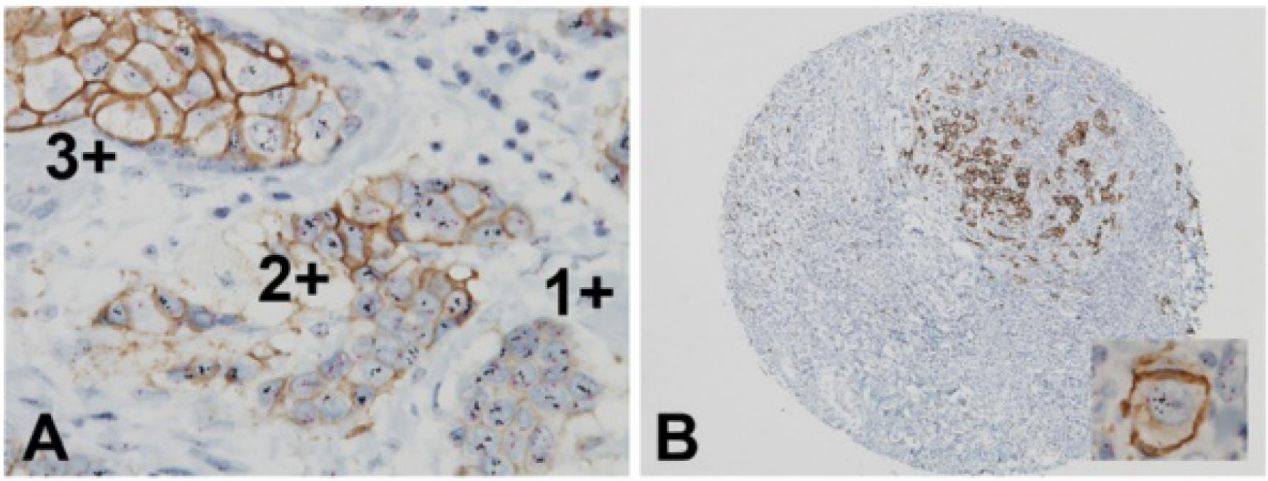


Figure 1. Results of HER2 gene-protein staining of FFPE breast cancer tissues exhibiting heterogeneity of HER2 positive tumor cell populations or isolated tumour cell populations. (A) The HER2 gene-protein assay demonstrated the heterogeneity of HER2 positive tumour cell populations in FFPE breast cancer 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.

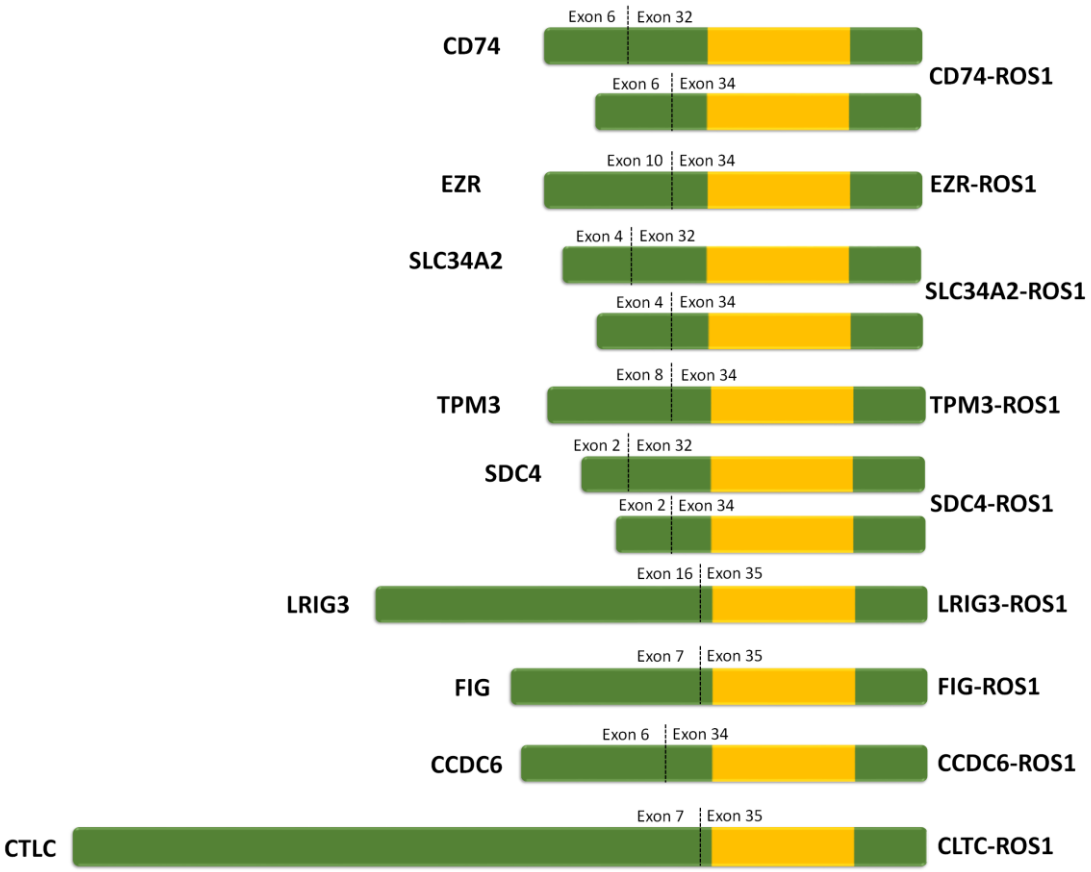
Tissue is still important

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 ROS1?

ROS1 was first identified in glioblastoma and cholangiocarcinoma[1,2]. ROS1 is a receptor tyrosine kinase (RTK) encoded by the ROS1 gene located on chromosome 6p22. Genetic rearrangements can occur with many different partner genes. The commonly reported partners are CD74, SLC34A2, FIG1, LRIG3, SDC4, SLCA2, TMP and EZR.

ROS1 Fusions Associated with Lung Adenocarcinoma



Schematic representation of the ROS1 fusions. The tyrosine kinase domain of ROS1 is depicted in yellow.

1. Proc Nat Acad Sci USA 1987 84 p9270-4
2. PLos One 2011 6:e 15640

The Role of ROS1 in Cancer

While ROS1 gene rearrangements were first identified in glioblastoma and cholangiocarcinoma[1,2], rearrangements have also recently been discovered in lung cancer[3], gastric cancer[4], colorectal cancer[5], ovarian cancer[6] and angiosarcoma[7].

Also found in non-small cell lung cancer (NSCLC), treatment of patients with Crizotinib has been found to be highly effective[8]. However, ROS1 rearrangements are reported to only be present in 1-2% of NSCLC cases.

ROS1 Assessment

Typically ROS1 translocations or fusions are assessed by Fluorescence in situ hybridisation (FISH). These are available from a variety of vendors including (but not limited to):

- Abbott Molecular Inc - Vysis
- Leica Biosystems - Kreatech
- Agilent Technologies - SureFISH
- ZytoVision – ZytoLight®
- CytoCell (ROS1 and ROS1 *Plus* Break Apart)

1. Proc Nat Acad Sci USA 1987 84 p9270-4
2. PLos One 2011 6:e 15640
3. Cell 2007 131 p1190-203
4. Cancer 2013 119 p1627-35
5. Mol Cancer Res 2014 12 111-8
6. PLos One 2011 6:e 28250
7. PLos Genetics 2013 9:e 1003464
8. Oncologist 2012 17 1351-75
9. J Thorac Oncol. 2017 Nov; 12(11): 1611–1625

Recently, monoclonal antibodies for immunohistochemistry (IHC) that are effective in formalin-fixed, paraffin-embedded specimens, have entered the market. These include:

- Clone D4D6 from Cell Signalling Technology Inc.
- Clone SP384 from Roche Ventana
- Clone MRQ-68 from Cell Marque (Sigma Aldrich)

ROS1 Analyte Control

The product consists of two cell lines: one with a SLC34A2-ROS1 translocation and resulting fusion protein. The second has no translocation and therefore no ROS1 fusion protein expressed. ROS1 Analyte Control is sold in two formats: pre-prepared slides (Figure 2) or as a cell microarray (CMA) paraffin wax block (Figure 3).

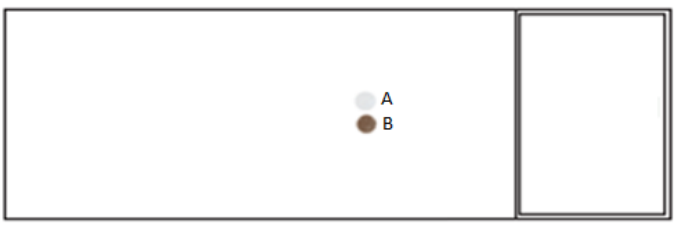


Figure 2: Cell Line Control Slide

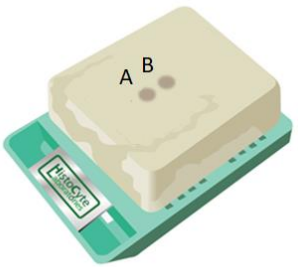


Figure 3: CMA block

Our CMA block provides the most cost effective solution for clinical histology laboratories and other high volume centers. They have been purposely designed to fit seamlessly into the work flow of the laboratory.

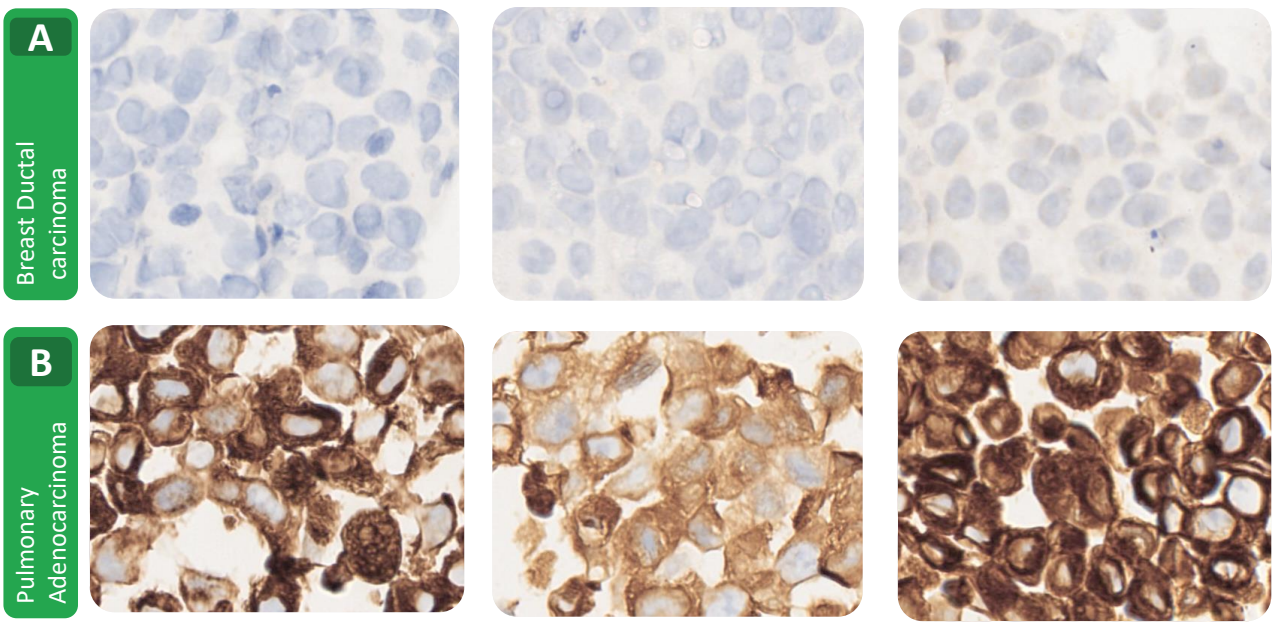
Our pre-prepared slides offer a ready-to-go alternative that saves time in preparation. These are ideal for one-off assessments, research laboratories and preliminary product trials.

ROS1 Analyte Control

The expression patterns of the 2 cell lines for ROS1 IHC and FISH are summarised below in the table.

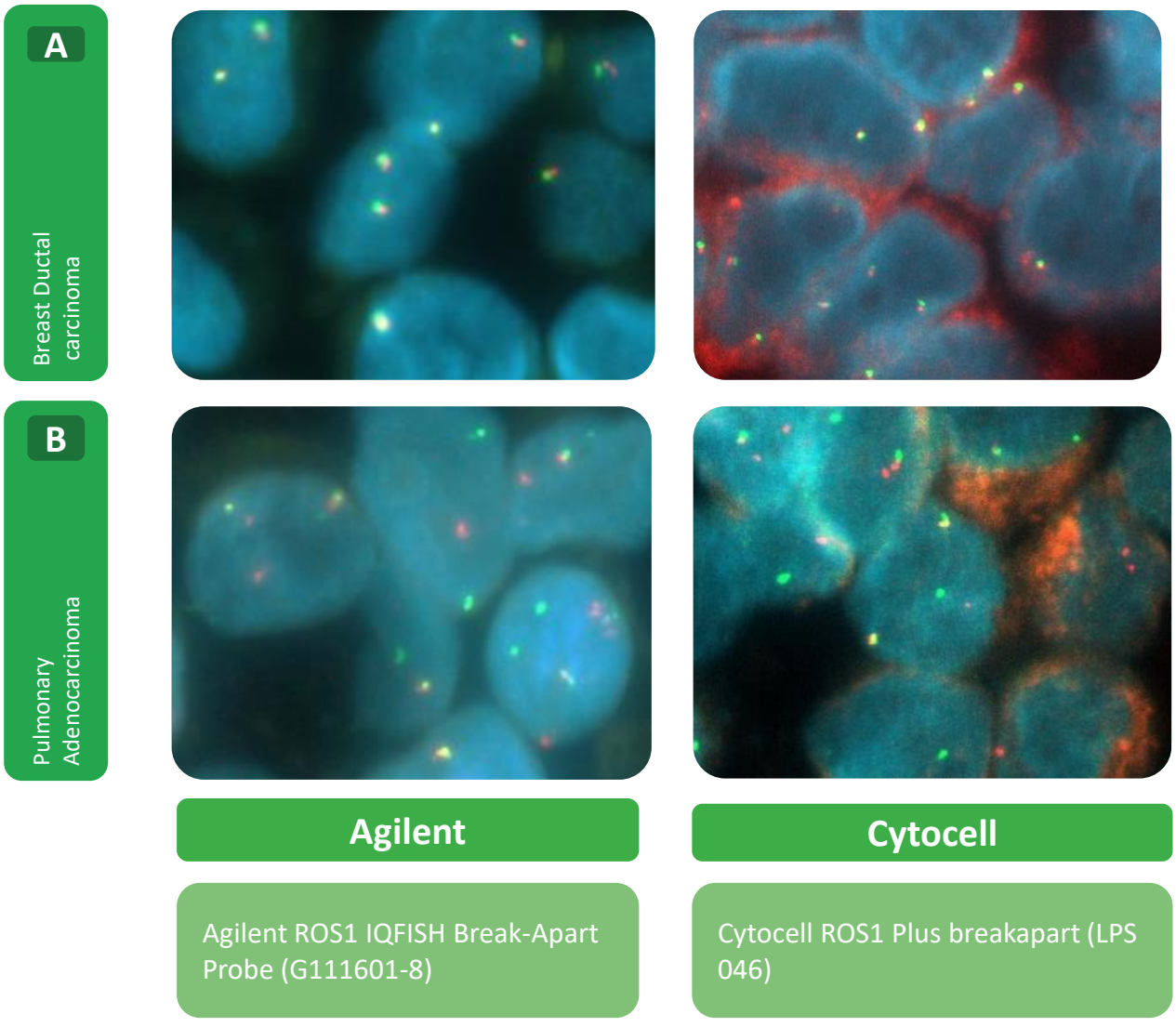
	Cell Lines	ROS1 IHC	ROS1 FISH
A	Breast Adenocarcinoma	Negative	Fusion signals
B	Lung adenocarcinoma	Positive	Break apart signals

ROS1 Analyte Control - IHC



CST D4D6®	Roche SP384	MRQ-68
Cell Signalling Technologies Clone D4D6® on the Ventana BenchMark ULTRA with VENTANA OptView. (Dil. 1/160) With Amplification	RTU SP384 (790-6087) on the Ventana BenchMark ULTRA. Standard protocol.	MRQ-68 RTU on the Ventana BenchMark ULTRA. Standard protocol.

ROS1 Analyte Control - FISH



The ROS1 Analyte Control while not providing a low or mid fusion protein expressing cell core does provide a cost effective positive/negative control.

For those requiring a control with more sensitivity and range of expression the ROS1 Analyte Control^{DR} was developed.

ROS1 Analyte Control^{DR}

ROS1 Analyte Control^{DR} contains an additional cell line with low expression of ROS1 (see figures 4 and 5). The low expressing glioblastoma cell line contains the FIG-ROS1 fusion, also known as GOPC-ROS1 (Golgi-associated PDZ and coiled-coil motif-containing protein). This is an interstitial deletion where the 5' end is lost rather than translocated as seen with SCL34A2-ROS1 where the 5' end resides elsewhere on another chromosome[1]. This is important as the kinase domain of the ROS1 protein is encoded at the 3' end. FIG-ROS1 has caused some controversy as to whether it is a viable target for ROS1 therapy[2]. However, other groups have demonstrated that it is affected by tyrosine kinase inhibitors in xenograft models. It may be in the former study that the overriding oncogenic driver was not FIG-ROS1[1].

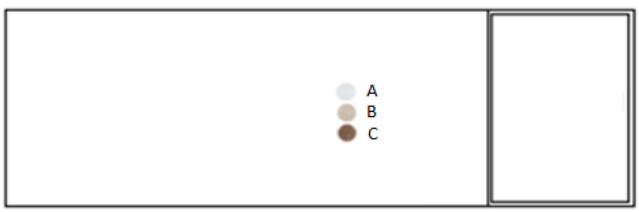


Figure 5. Cell Line Control Slide

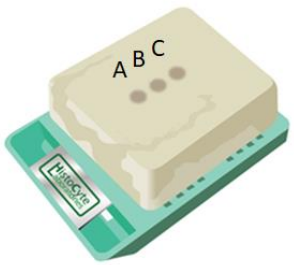


Figure 4. CMA/Block

Expression pattern for the three cell lines for ROS1 IHC and FISH are summarised below in the table.

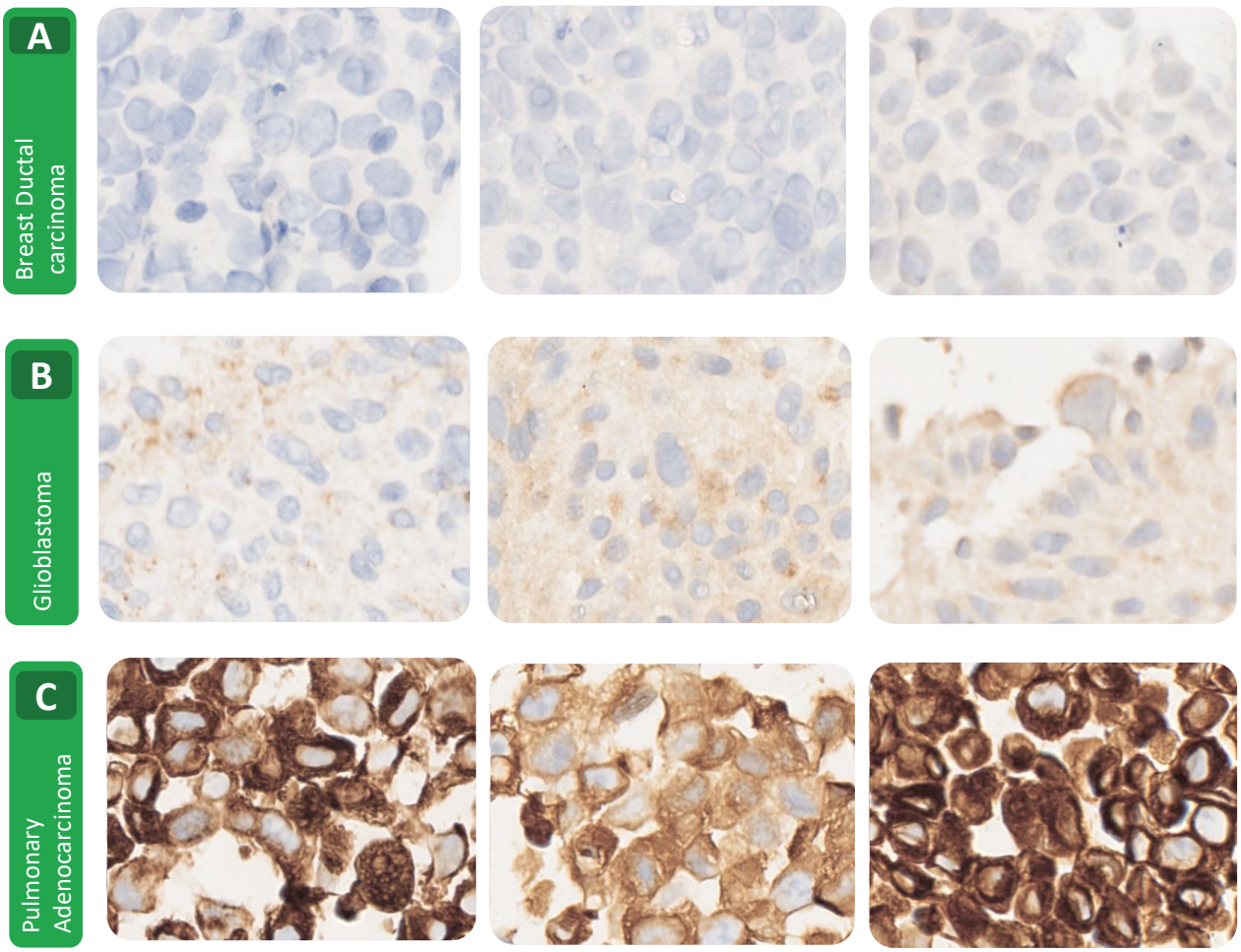
	Cell Lines	ROS1 IHC	ROS1 FISH
A	Breast Adenocarcinoma	Negative	Fusion signals
B	Glioblastoma	Low	Loss of ROS1*
C	Lung adenocarcinoma	Positive	Break apart signals

*results are dependent on the assay used

1. Clin Cancer Res; 18(16) August 15, 2012
2. Clin Cancer Res. 2013 August 1; 19(15): 4040–4045

Regardless it appears as a promotor FIG is not as potent as other translocation partners as the fusion protein is expressed at very low levels. Antibodies have to be run at relatively high concentrations (as we have found during development) or signal amplified in some way[1].

ROS1 Analyte Control^{DR} - IHC

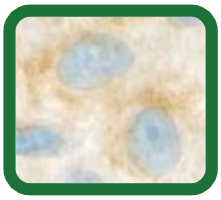


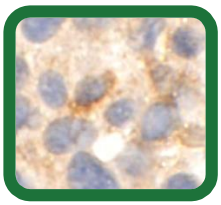
CST D4D6®	Roche SP384	MRQ-68
Cell Signalling Technologies Clone D4D6® on the Ventana BenchMark ULTRA with VENTANA OptView. (Dil. 1/160) With Amplification	RTU SP384 (790-6087) on the Ventana BenchMark ULTRA. Standard protocol.	Cell Marque RTU MRQ-69 on the Ventana BenchMark ULTRA. Using the SP384 Standard protocol.

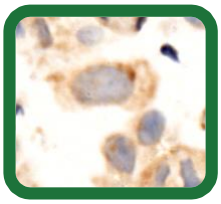
Images taken at x40 Hamamatsu NanoZoomer.

1. Virchows Arch (2016) 469:489–503

FIG-ROS1 Expressing Cell Line







The staining in the low expressing cell line that contains the FIG-ROS1 translocation/deletion can be quite subtle. It is a good indicator of the assay's level of sensitivity. The GOPC-ROS1 or FIG-ROS1 fusion protein localises in the Golgi typically giving a stronger perinuclear or more intense foci around the nuclei. The images here show examples of this. Otherwise the staining is a general low cytoplasmic staining. It is therefore important that while blushing may occur in the negative control, it should never exceed that seen in the low cell core.

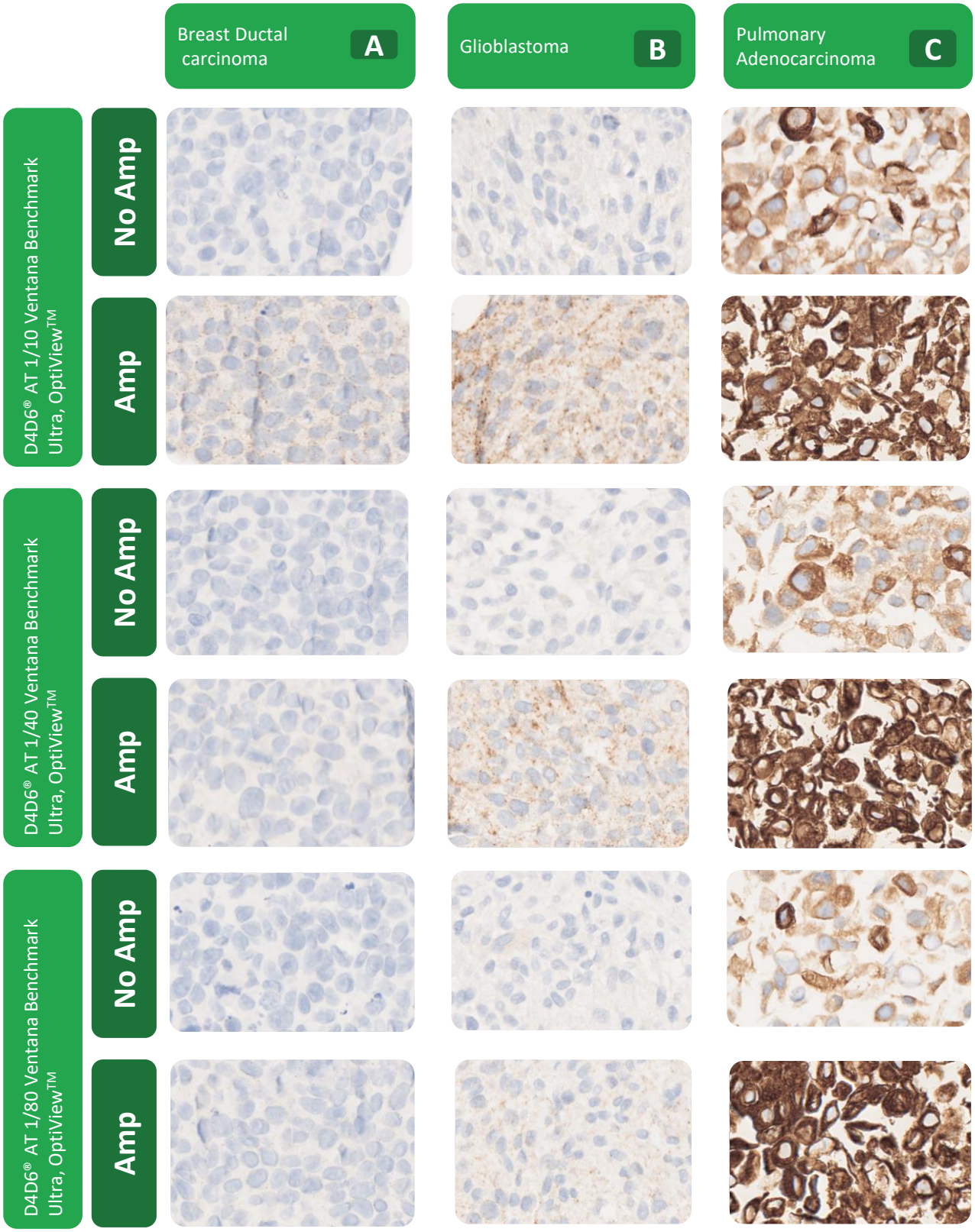
Assay Sensitivity

The antibodies available differ in their sensitivity. The D4D6® clone was one of the first and most widely adopted. The following shows the antibody titrated with and without amplification. This demonstrates how sensitive the clone is in detecting the low expression of the FIG-ROS1 fusion protein. Bubendorf *et al*[1] had described using amplification to increase the clones sensitivity to create good correlation with FISH.

The following were titrations of D4D6® with and without amplification. Even at 1/10 without amplification on the Ventana Benchmak Ultra with OptiView™ cell line B was not convincing. With amplification staining was good to about 1/160, beyond this it was less robust across multiple batches.

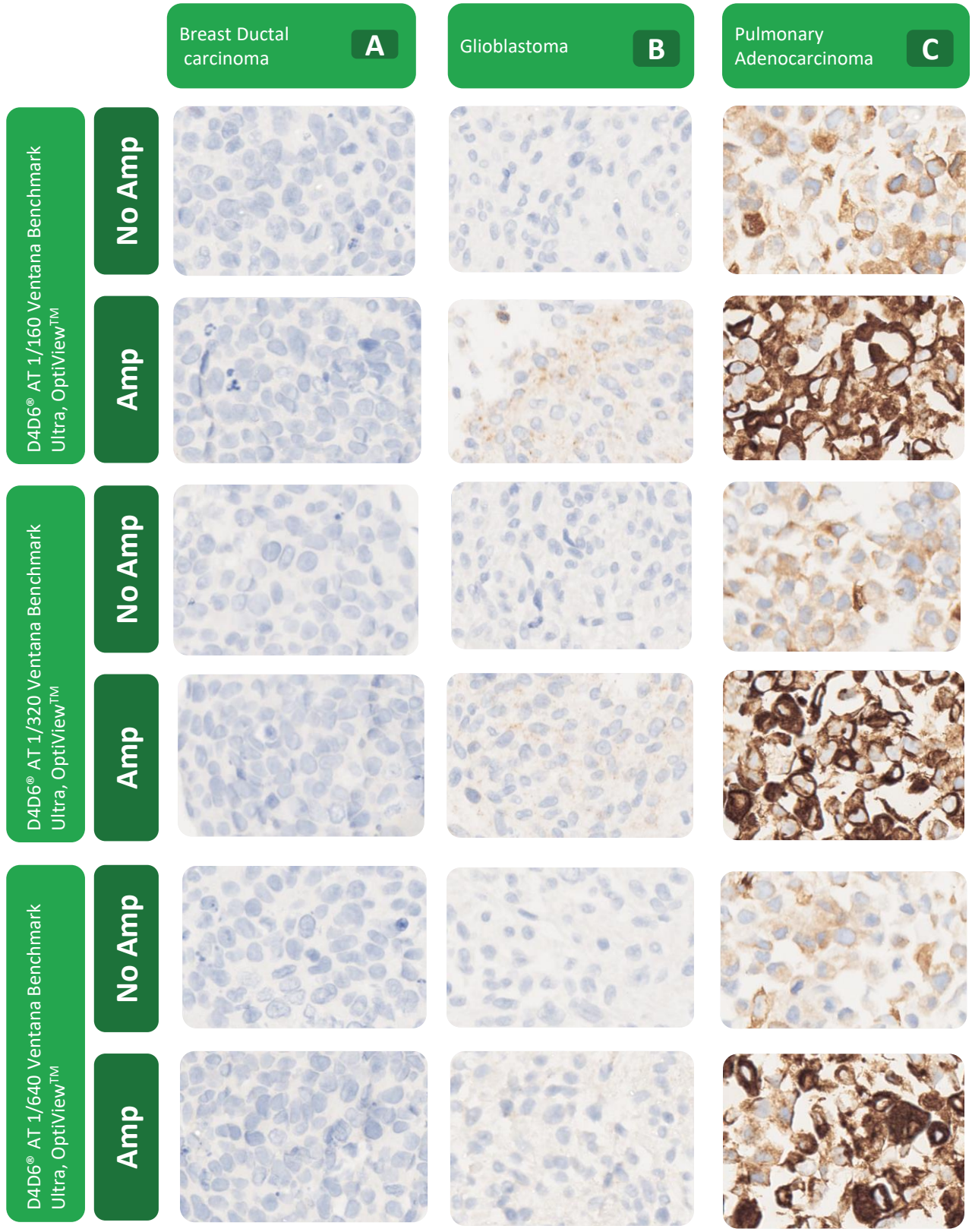
1. Virchows Arch (2016) 469:489–503

D4D6® Titration



Images taken at x40 Hamamatsu NanoZoomer.

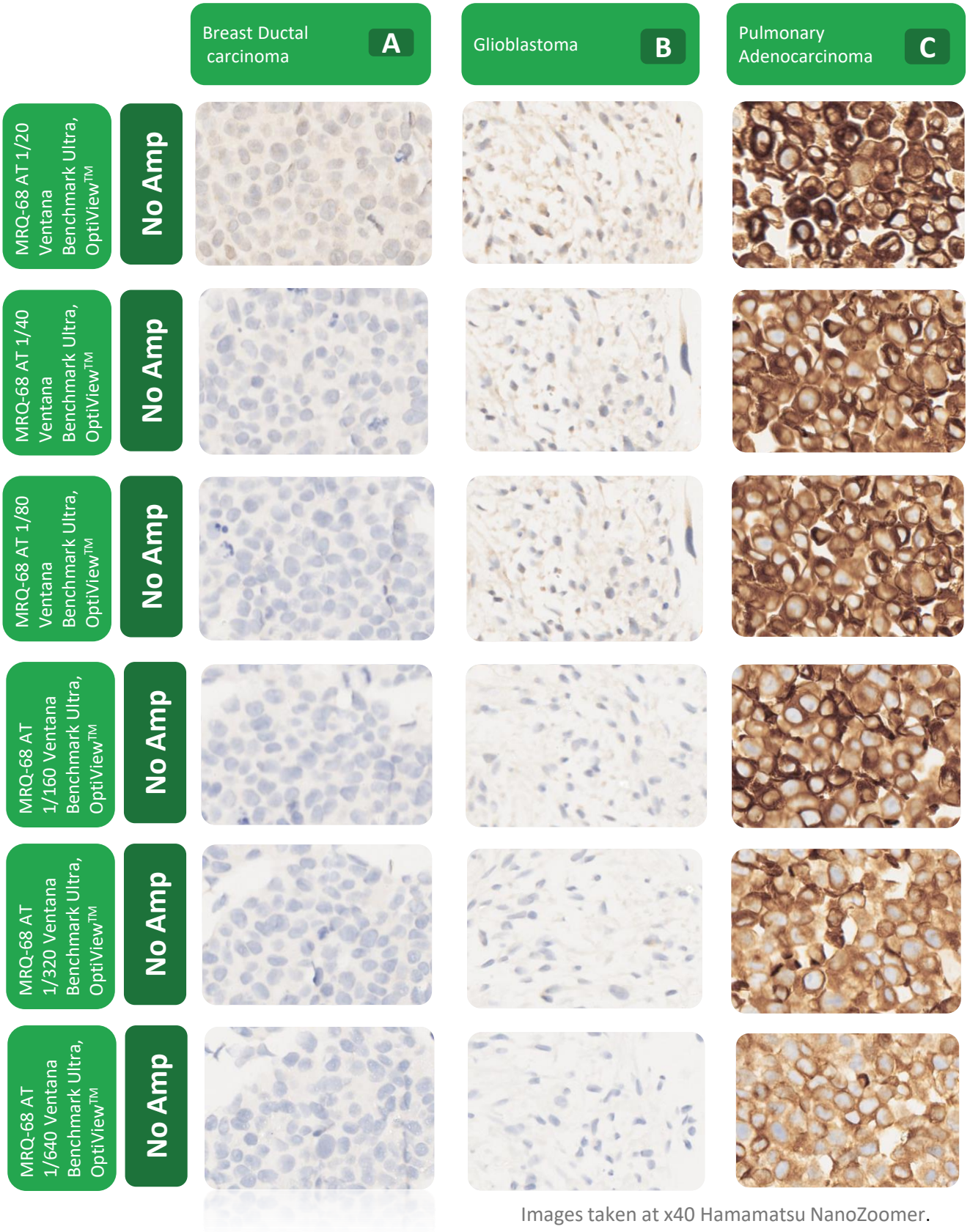
D4D6® Titration



Images taken at x40 Hamamatsu NanoZoomer.

MRQ-68 Titration

Clone MRQ-68 from Cell Marque titration without amplification.



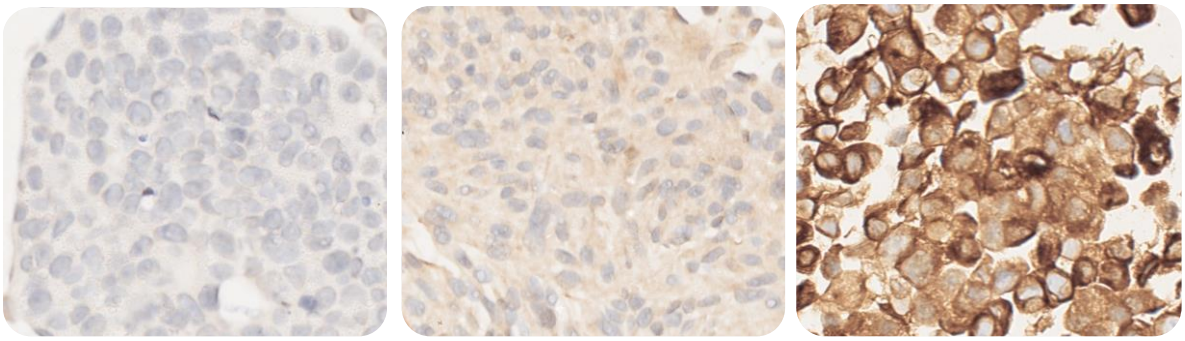
Images taken at x40 Hamamatsu NanoZoomer.

MRQ-68 Titration

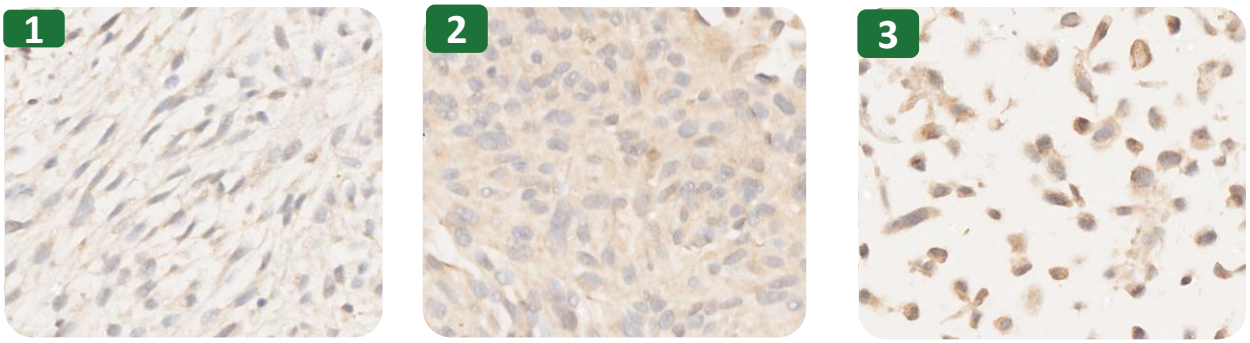
Greatest reproducibility on ROS1 Analyte Control[DR] with clone MRQ-68 was seen at 1/30.

MRQ-68 AT 1/30 Ventana Benchmark Ultra, OptiView™

Breast Ductal carcinoma A	Glioblastoma B	Pulmonary Adenocarcinoma C



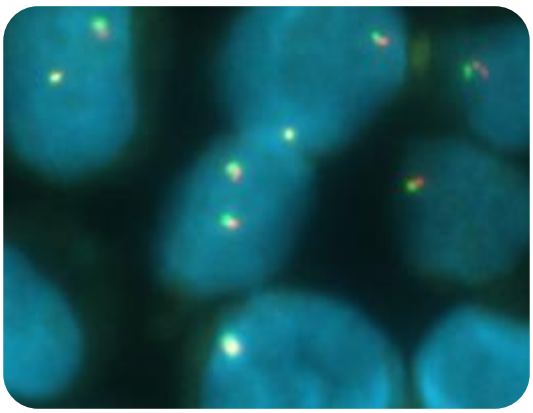
Cell B Mixed Morphology

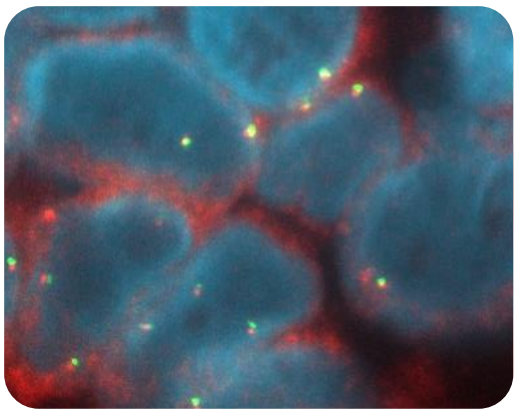


Tested 1/30 with MRQ-68: Image 1 the cells have a spindle form, there is less cytoplasm to view and as such the staining in the cell prep appears lower than image 2. Image 2 is epithelioid with more cytoplasm giving more surface area to stain. Therefore this looks stronger than image 1. Image 3 the cell preparation is less dense with mixed morphology. The cells appear small with a shrunken cytoplasm. The net effect is that the cytoplasm is concentrated giving a more intense staining to the cells. These morphologies can appear in the same batch depending on how the cells develop. In each case they are appropriately positive.

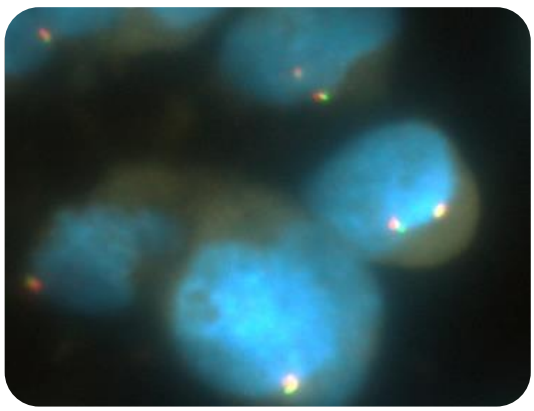
ROS1 Analyte Control - FISH

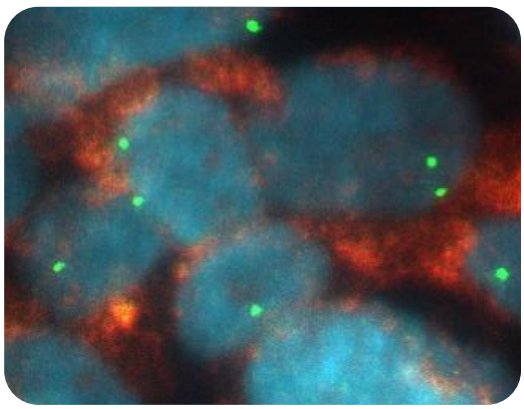
A Breast Ductal carcinoma



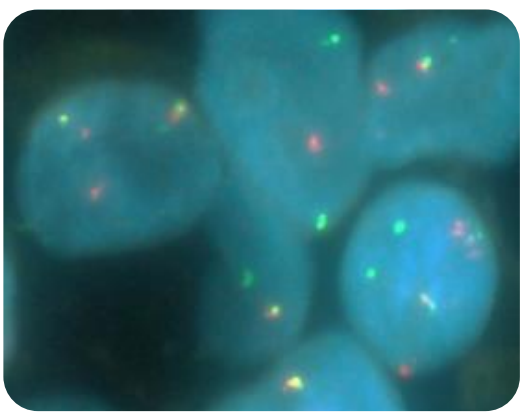


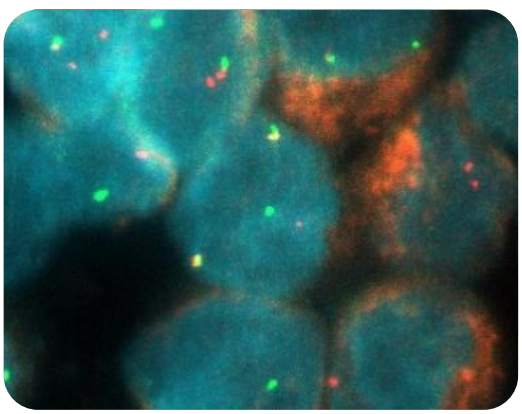
B Glioblastoma





C Pulmonary Adenocarcinoma





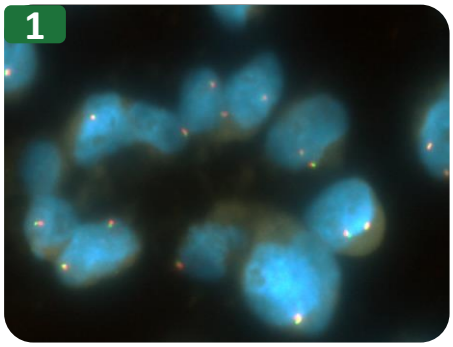
Agilent	Cytocell
Agilent ROS1 IQFISH Break-Apart Probe (G111601-8)	Cytocell ROS1 Plus breakapart (LPS 046)

NB: The Agilent probes are unable to detect the ROS1 deletion in cell line B, see page 18 for further information.

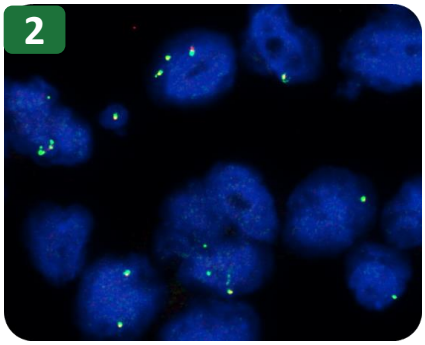
ROS1 Analyte Control - FISH

It is important to note that FIG-ROS1 is not identified by all FISH assays. Agilent ROS1 FISH is unable to detect the deletion demonstrating overlapping signals (see image 1 below). FIG-ROS1 is an interstitial deletion that gives rise to a fusion protein hence it is detected by IHC optimised appropriately.

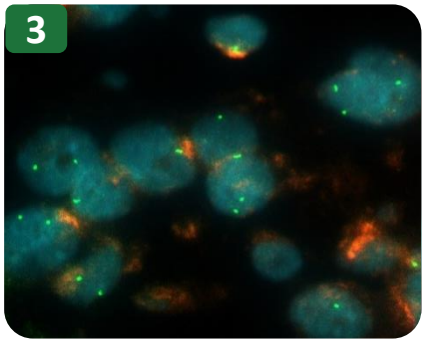
There are at least two assays (see image 2 and 3) that can determine whether there is a deletion, this has been documented previously and is mentioned, along with the specificity of other probes, in the International Association for the Study of Lung Cancer Atlas of ALK and ROS1 Testing in Lung Cancer, 2nd Edition[1].



ROS1 IQFISH Break-Apart Probe (G111601-8)



ZytoLight ® SPEC ROS1 Dual Color Break Apart Probe (Z-2144-50)



CytoCell ROS1 Plus Breakapart Probe (LPS 046/LPS 046-S)

The CytoCell and Zytovision assays were run on the cells to confirm this. In addition all three cell lines were tested by Next Generation Sequencing, the following results were reported:

Cell line A: No fusion detected
Cell line B: GOPC(8)-ROS1(35)
Cell line C: SLC34A2(4)-ROS1(32)

Ion Torrent Oncomine Focus library preparation kits and Ion Torrent S5 sequencers with data analysed using Ion Reporter v 5.10.3.0

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 SCL34A2-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

HistoCyte Laboratories
Quality in Control