medac

HistoCyte Laboratories

Quality in Control

Estrogen Receptor Control^{DR}

Product Introduction

Product Codes:

HCL029, HCL030 and HCL031

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Issue 1. 180510

Contents

Product Name	Format	Code
Estrogen Receptor Analyte Control^{DR}	Slide (2)	HCL026
	Slide (5)	HCL027
	Block	HCL028

(For research use only)

Introduction to Estrogen Receptor

What is it?

Estrogen Receptor (ER) is a member of the Nuclear Hormone Receptors (NHR). These play a diverse role in the cellular processes acting as transcription factors.[1] Their activity is modulated by steroid hormones including Estrogens. Estrogens are a class of hormones that include estrone, estriol and estradiol (E2).[2]

Estrogen freely passes the cellular membrane binding monomeric ER in the nuclei. This interaction leads to conformational change in ER causing receptor dimerization. This ligand/receptor complex is known as the estrogen response element (ERE) and interacts directly with the co-activator proteins and polymerases that result in the transcription of pro-proliferative effectors.[3]

Utility

Estrogen receptor has long been a target for treatment in breast cancer with the greatest impact occurring with the introduction of Tamoxifen.[4] Tamoxifen is a selective ER modulator (SERM) as it binds to ER causing dimerization yet does not induce the same recruitment of co-activators as the estrogens do. Newer therapies such as the aromatase inhibitors (AI) have demonstrated a greater effectiveness in both the adjuvant and metastatic setting.[5] Initially ER status was determined by ligand binding assays, however, this was superseded by immunohistochemistry (IHC) and determined to be better at predicting response.[6]

1. Ribeiro RC, Kushner PJ, Baxter JD. The nuclear hormone receptor gene superfamily. *Annu Rev Med*. 1995;46:443–453.
2. Gruber CJ, Tschugguel W, Schneeberger C, Huber JC. Production and actions of estrogens. *N Engl J Med*. 2002;346:340–352
3. Klinge, C.M. (2000) Estrogen receptor interaction with co-activators and co-repressors. *Steroids*, 65, 227-251
4. Jordan C: Historical perspective on hormonal therapy of advanced breast cancer. *Clin Ther* 24:A3–A16, 2002 (suppl A)
5. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Aromatase inhibitors in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet*. 2015 Oct 3;386(10001):1341-1352.
6. Harvey JM, Clark GM, Osborne CK, et al: Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J Clin Oncol* 17:1474–1481, 1999

ER immunohistochemistry

The tests

There are multiple clones available on the market. The principle of their use is to determine the expression of nuclear ER. The assays differ in the detection chemistry as well as species of the primary antibody. The most commonly used are:

- Agilent (Dako):
  - 1D5 mouse monoclonal[C/R]
  - EP1 rabbit monoclonal[C/R]
- Cell Marque Inc (Merck KGaA)
  - SP1 rabbit monoclonal[C]
- Leica Biosystems (Novocastra):
  - 6F11 mouse monoclonal[C/R]
- Roche (Ventana):
  - SP1 rabbit monoclonal[R]

Scoring ER

There are a number of methods, primarily:

1. **Percentage**: essentially the percentage of the specimen that is positive: 0%: No cells stained to, 100%: All cells stained. Samples are considered ER positive if they demonstrate as little as 1% of cells staining.
2. **Allred Score** (named after the inventor of the method) also known as the Quick Score: is based on the intensity and the proportion of cells staining. The combined scores give an overall value from 0-8. The values for each are derived as follows:

[C] Available as a concentrate
[R] Available as in a ready to use format

The proportion of tumor nuclei showing positive staining is scored as follows:

0%	= 0
<1%	= 1
1–10%	= 2
11–33%	= 3
34–66%	= 4
67–100%	= 5

This is combined to a score for the overall intensity of the staining in the cells:

Negative (no staining of any nuclei at high magnification)	= 0
Weak (only visible at high magnification)	= 1
Moderate (readily visible at low magnification)	= 2
Strong (strikingly positive at low magnification)	= 3

H-Score

Or Histo score can be applied to any tumor sample. The result is determined by the percentage of cells staining at each intensity. Intensity range is based on 0 (none), 1+ (weak), 2+ (moderate), 3+ (strong). The H-scores are then determined by:

[1x(% cells 1+) + 2x(% cells 2+) + 3x(% cells 3+)]

Quality Control

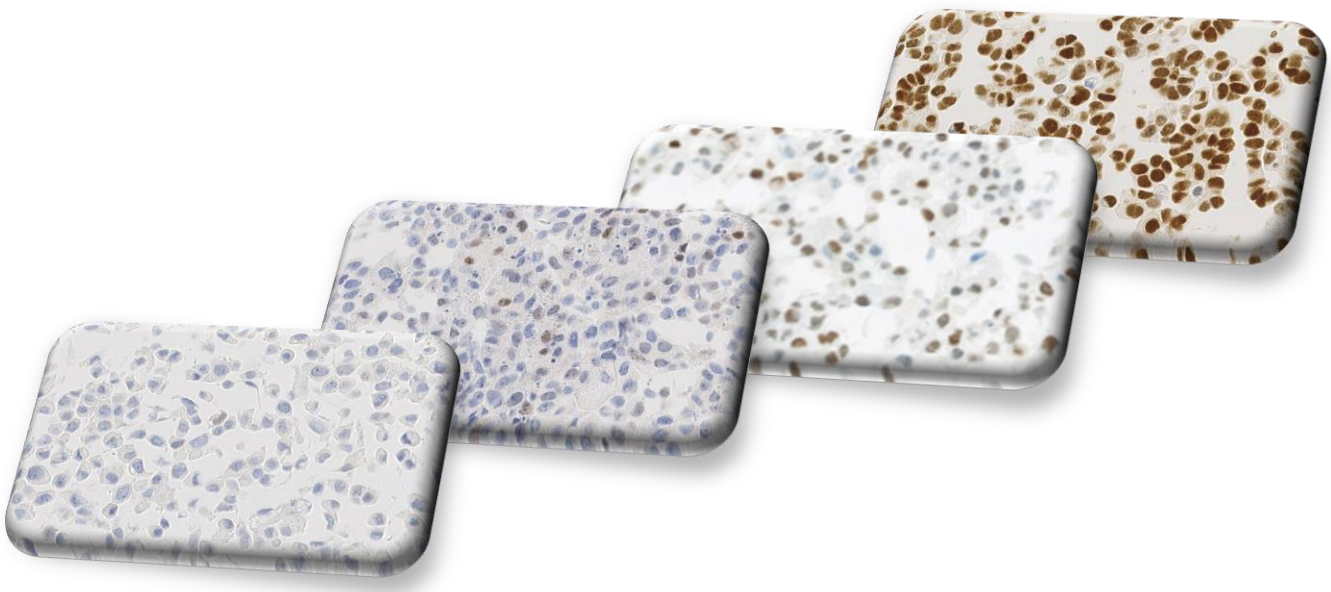
One of the requirements of quality standardization is the appropriate use of controls. These need to be robust enough for IHC and in situ hybridization (ISH), be reproducible and cost-effective. Additionally, the control material should be consistent from batch to batch and throughout the block it is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

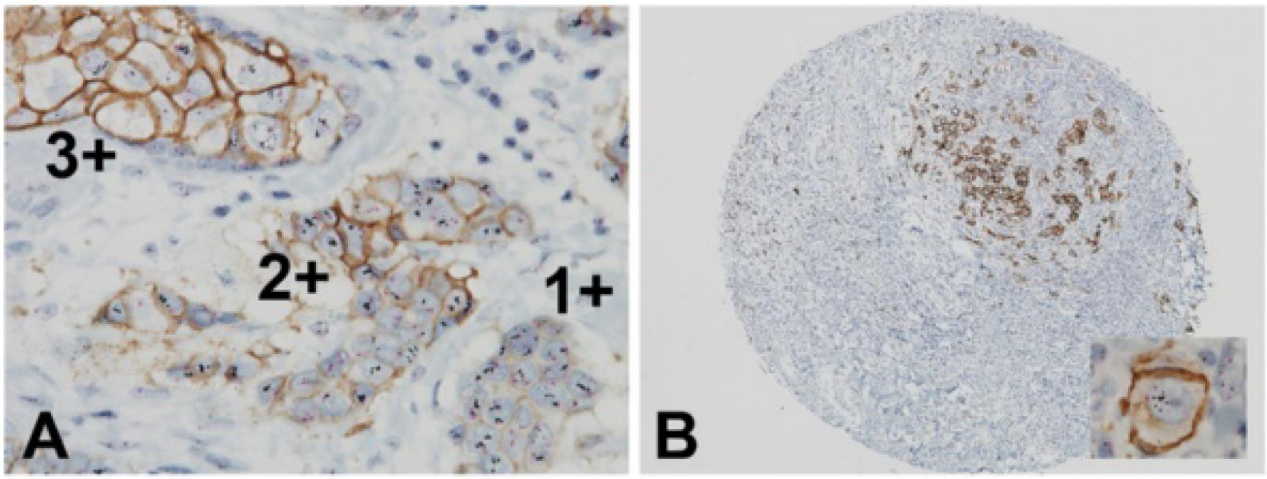


Figure 1. Results of HER2 gene-protein staining of FFPE breast cancer tissues exhibiting heterogeneity of HER2 positive tumor cell populations or isolated tumour cell populations. (A) The HER2 gene-protein assay demonstrated the heterogeneity of HER2 positive tumour cell populations in FFPE breast cancer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

Tissue is still important

It is important to remember that these 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.

ER Analyte Control^{DR}

The **ER Analyte Control^{DR}** is sold in two formats: as pre-prepared slides (Figure 2) or as a cell microarray (CMA) paraffin wax block (Figure 3).

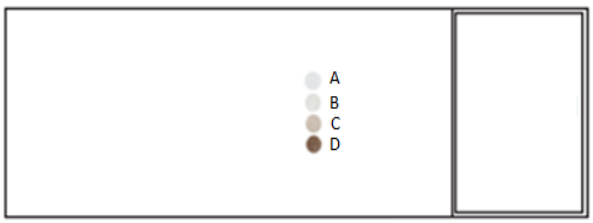


Figure 2: Cell Line Control Slide

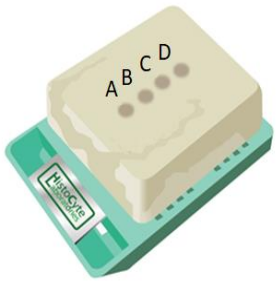


Figure 3: CMA block

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.

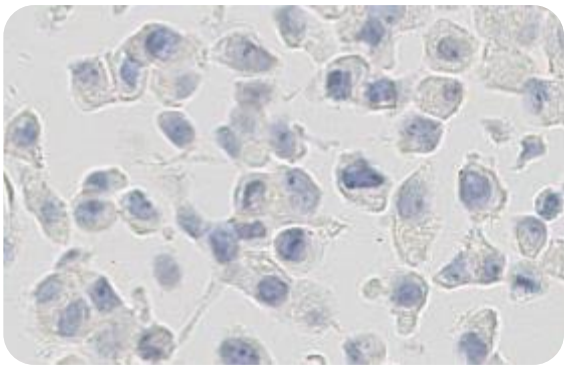
Our pre-prepared slides offer a ready-to-go alternative that saves time in preparation. These are ideal for one-off assessments, research laboratories and preliminary product trials.

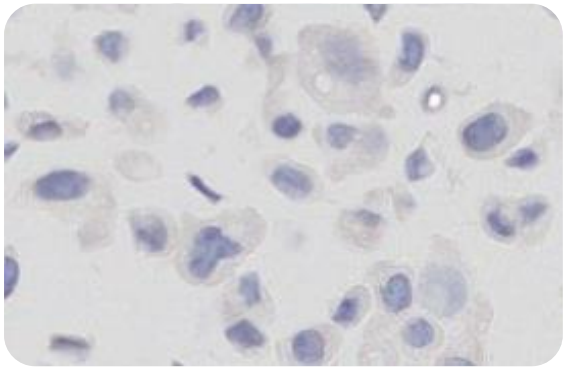
The expression patterns of the 4 cell lines for ER are shown below:

Cell Lines	IHC for Estrogen Receptor
A	Negative
B	Low
C	Intermediate
D	High

ER Analyte Control^{DR} IHC

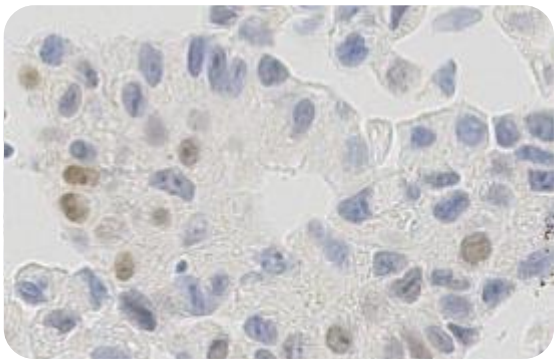
A Breast adenocarcinoma

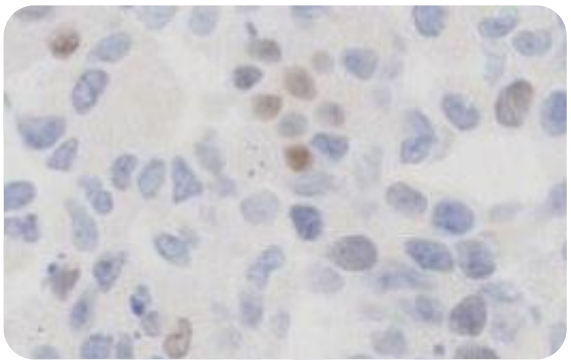




Negative

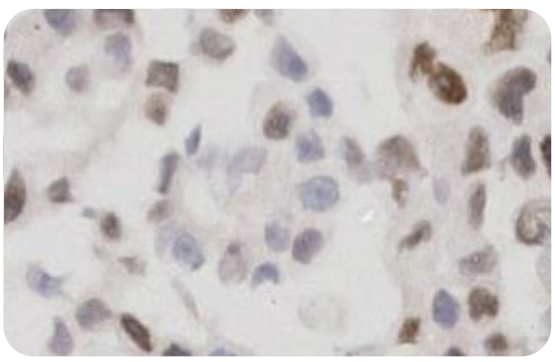
B Urothelial carcinoma

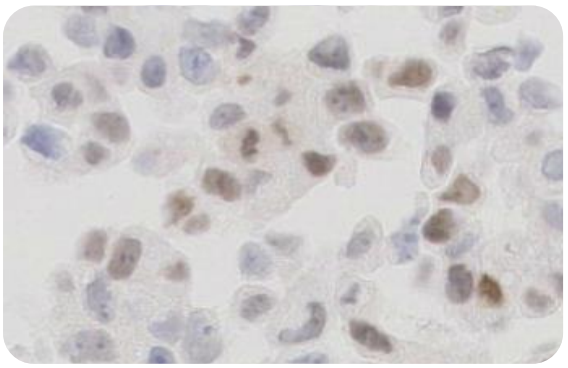




Low

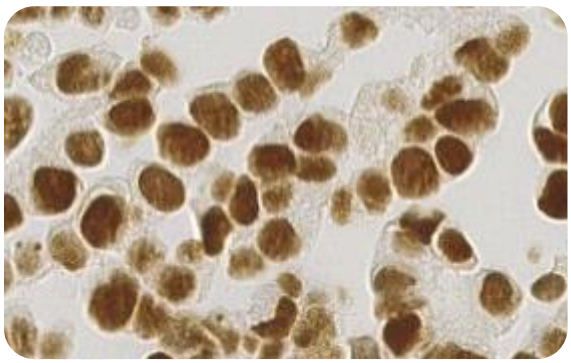
C Non-small cell lung carcinoma

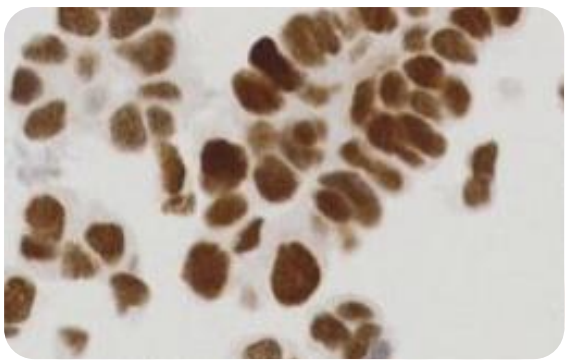




Intermediate

D Breast adenocarcinoma

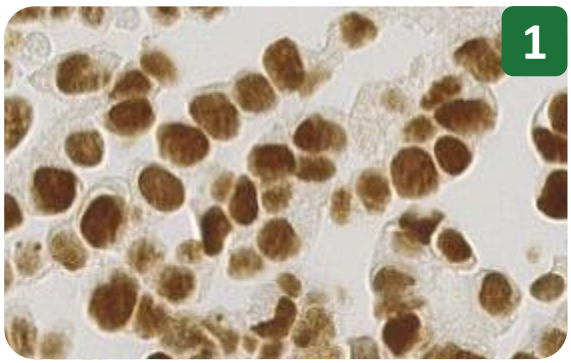




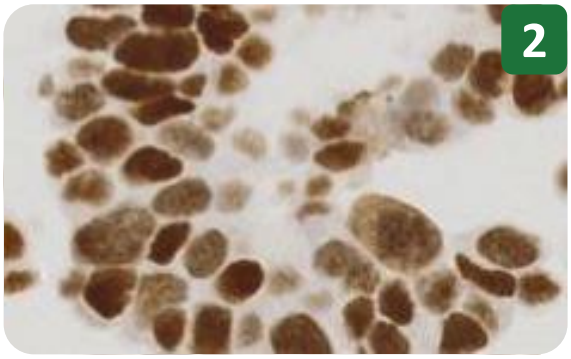
High

Roche/Ventana	Cell Marque Inc.
CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody. Standard protocol (CC1 64mins, Primary incubation 16 mins) (Product Code: 790-4324)	**Estrogen Receptor (SP1) Rabbit Monoclonal Antibody; Used at 1/75* on the Ventana Benchmark Ultra. (same protocol as the Ventana SP1 RTU) (Product Code: 249R-16)**

High ER Cell Line Staining



Typical staining demonstrates >90% of the cells with strong nuclear staining. Standard protocol for SP1 RTU on the Ventana/Roche.



If the ready to use antibody is left on longer than normal the staining is not much stronger as the antibody is likely exhausted. Image 2 shows SP1 RTU after a 60 minute incubation.

The effects of insufficient incubation of an optimised antibody concentration can be as detrimental as running an over diluted antibody for the “right” incubation period.

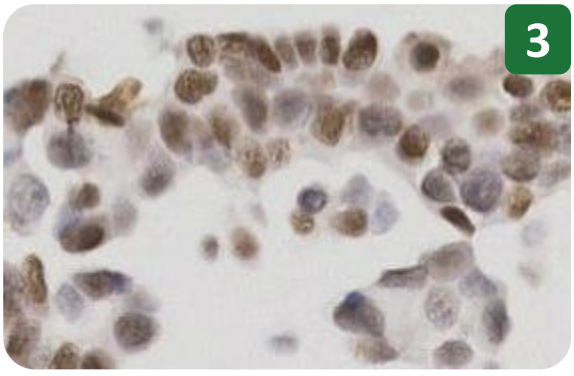
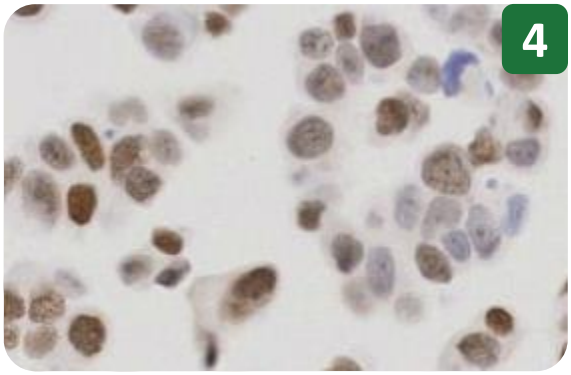
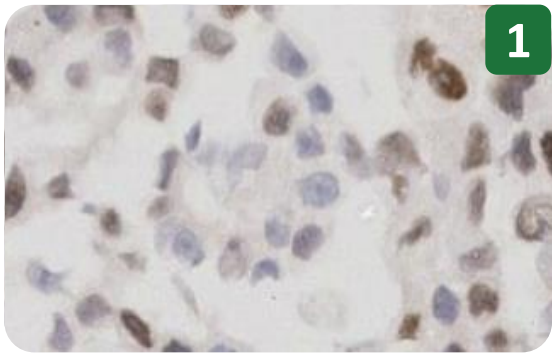


Image 3 shows SP1 staining on the Ventana using the standard protocol but with a 4 minute primary incubation. The antibody binding kinetics are not rapid enough for this to be effective.

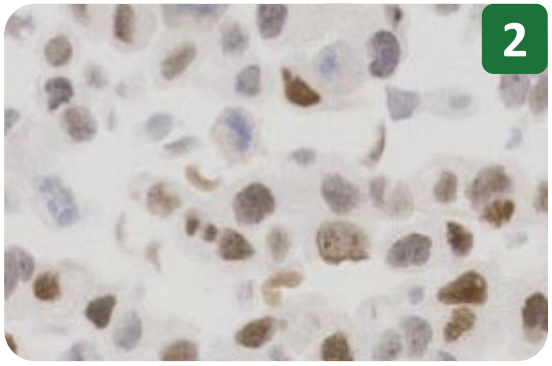


This result is similar for SP1 from Cell Marque if run at 1/300* dilution on the Ventana using the standard protocol. Here there is insufficient amount of antibody to saturate the antigen available.

Intermediate ER Cell Line Staining



Typical staining demonstrates >50% of the cells with weak to strong nuclear staining.



As seen with the high expressing cell line, excessive primary incubations with the RTU do not create over staining as the available antibody is likely exhausted.

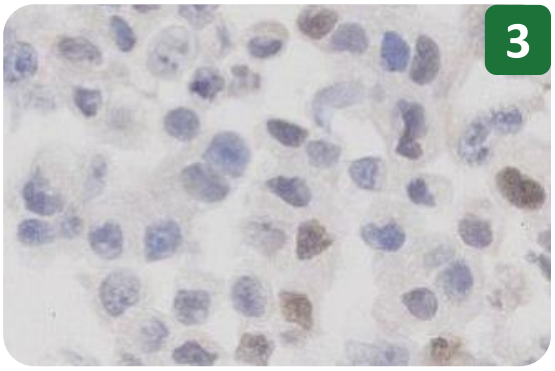
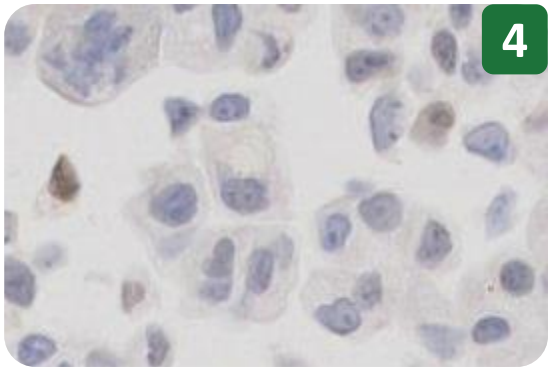
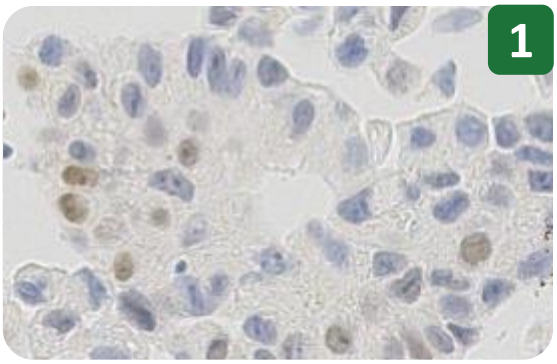


Image 3 shows SP1 staining on the Ventana using the standard protocol but with a 4 minute primary incubation. This is considerably weaker than the standard 16 minutes.

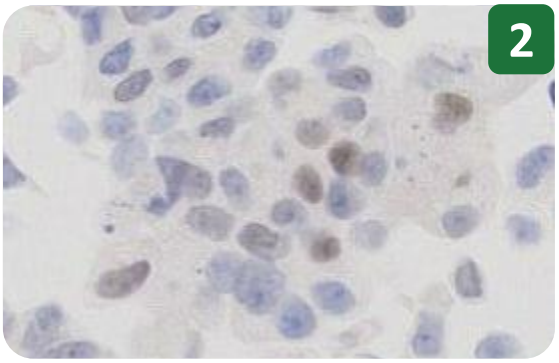


As with the high cell line the result is similar for SP1 from Cell Marque if run at 1/300* dilution on the Ventana using the standard protocol.

Low ER Cell Line Staining



Use of the standard protocol from Ventana/Roche (CC1 64 minutes, antibody incubation 16 minutes) typically demonstrates >5% of the cells with weak to moderate nuclear staining.



As seen with the other cell lines, excessive RTU primary antibody incubations do not create over staining as the available antibody is likely exhausted.

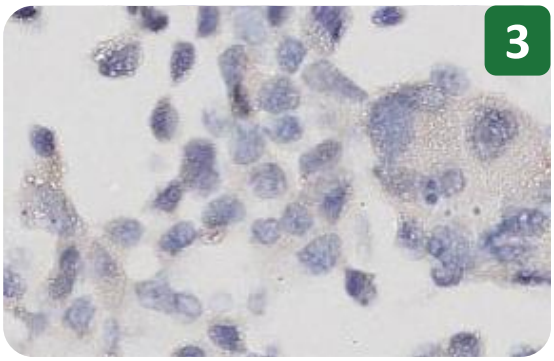
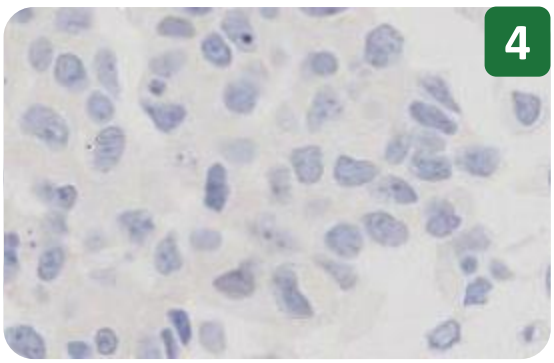
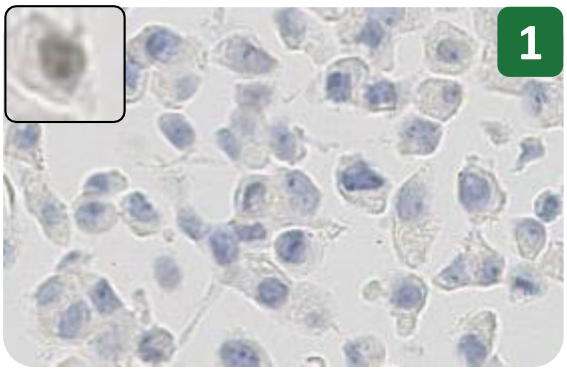


Image 3 shows SP1 RTU staining on the Ventana using the standard protocol but with a 4 minute primary incubation. This is negative with absence of any nuclear staining.

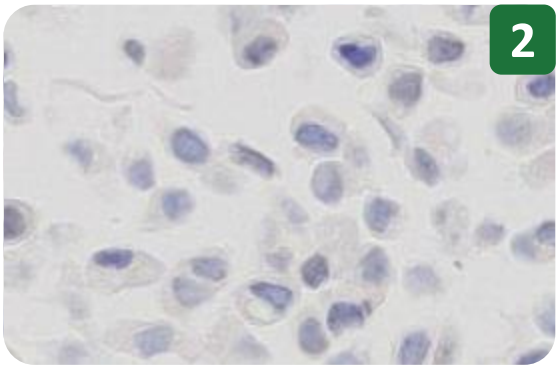


Similar negative result for SP1 from Cell Marque if run at 1/300* dilution on the Ventana using the standard protocol.

Negative ER Cell Line Staining



Typical staining pattern is absence of any nuclear staining. Occasionally a few nuclei will stain very weakly.



Excessive incubations with the RTU primary antibody do not create over staining. The Ab is optimised to a concentration that doesn't create staining.

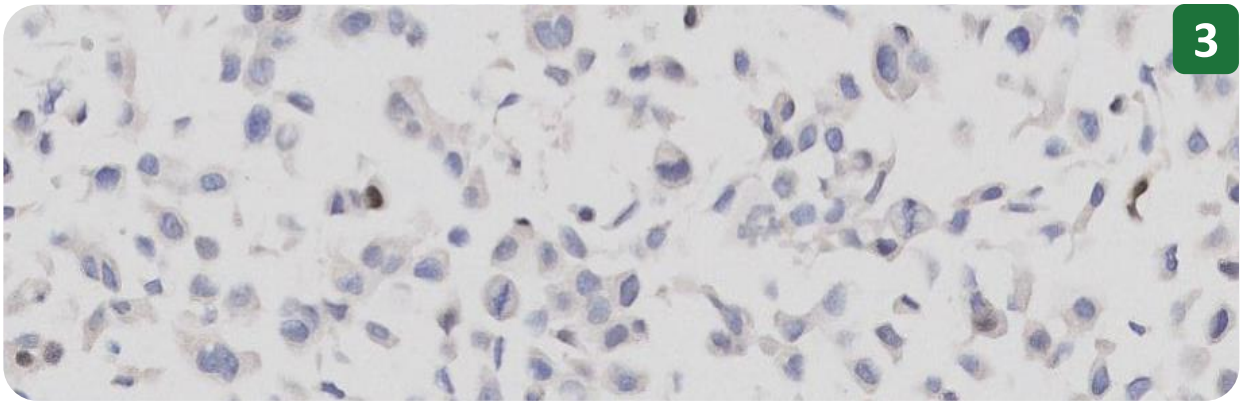
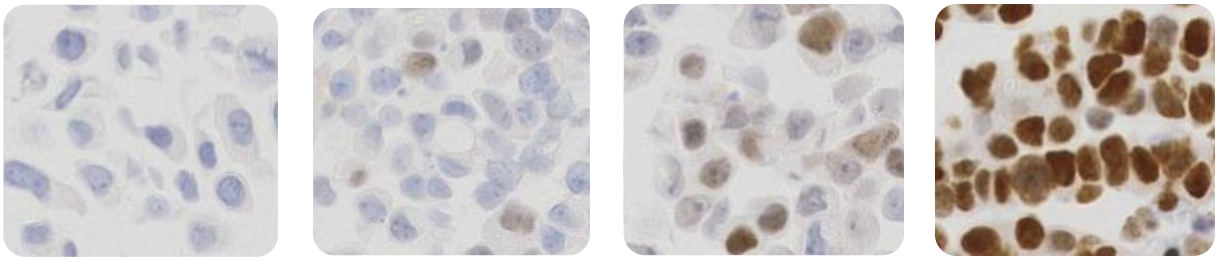


Image 3 shows staining using the Ventana platform and standard protocol with the SP1 clone from Cell Marque run at 1/12*. Excess antibody incubated for 16 minutes (standard protocol time) does force more staining than otherwise observed.

*Cell Marque SP1 dilutions described throughout are the final dilution on the slide. There is residual buffer on the slides with the Benchmark Ultra of ~280-300µl. Therefore adding 150µl to the slide results in a further 1/3 dilution of the aliquot. So for a 1/75 final dilution, an aliquot of 150µl at 1/25 is added to the slide.

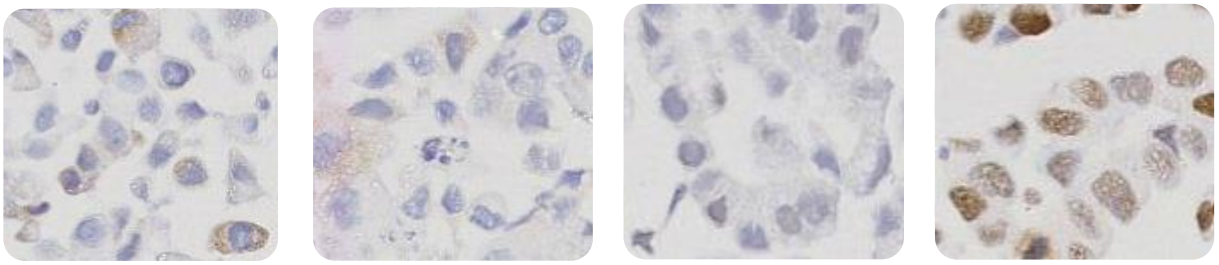
ER Analyte Control^{DR} Performance

CC1 retrieval for 92 minutes



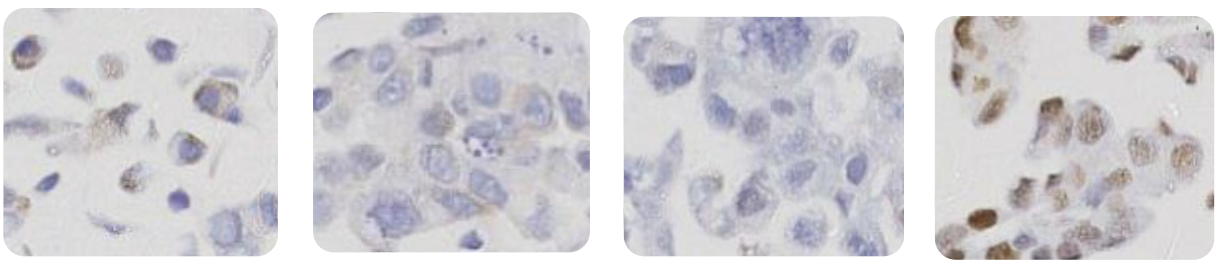
Over retrieval does not affect the overall performance of the IHC. Typically if the material is over retrieved the morphology is effected.

CC1 retrieval for 18 minutes



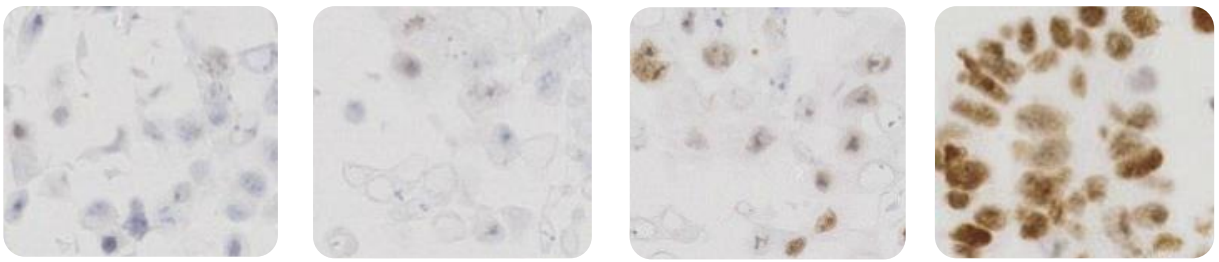
Under retrieved specimens are classically weaker but also the overall quality is affected. Additionally the negative cells acquire non-specific cytoplasmic staining.

No retrieval



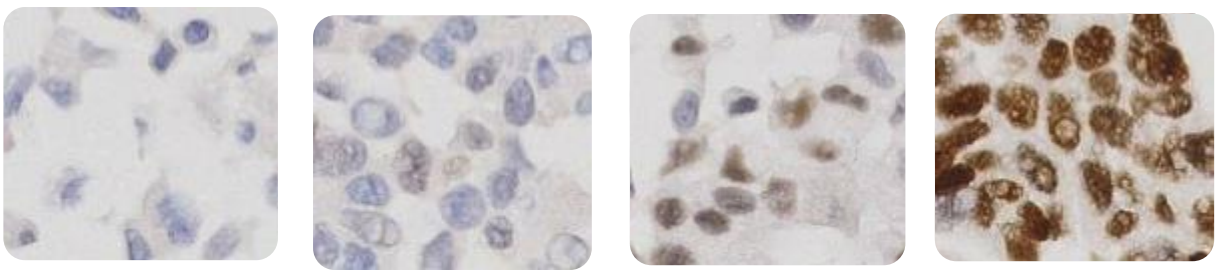
No retrieval causes non-specific staining similar to the under retrieved specimens, particularly in the negative cell line. Overall poor quality stain, the run has failed.

CC2 retrieval 64 minutes



CC2, pH6 distorts the morphology and fails to unmask the epitope satisfactorily.

No dewax



Despite no dewax the IHC has worked to some degree. This is because the slides are heated and repeatedly washed throughout the protocol on the Ventana, thereby removing the wax. However, it is not entirely effective leaving wax deposits affecting the result. This is clearly seen in the High expressing cell line.

Also Available From HistoCyte Laboratories Ltd

Product Name	Format	Code
HPV/p16 Analyte Control[DR] (Four cores 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-Lung Analyte Control (Two cores, positive and negative for the EML4-ALK translocation)	Slide(2)	HCL007
	Slide(5)	HCL008
	Block	HCL009
ALK-Lymphoma Analyte Control (Two cores, positive and negative for the NPM-ALK translocation)	Slide(2)	HCL010
	Slide(5)	HCL011
	Block	HCL012
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 Analyte Control[DR] (4 cores with a dynamic range of expression of PD-L1)	Slide(2)	HCL019
	Slide(5)	HCL020
	Block	HCL021
ROS1 Analyte Control (Two cores, positive and negative for ROS1 translocation)	Slide(2)	HCL022
	Slide(5)	HCL023
	Block	HCL024
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

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HistoCyte Laboratories
Quality in Control