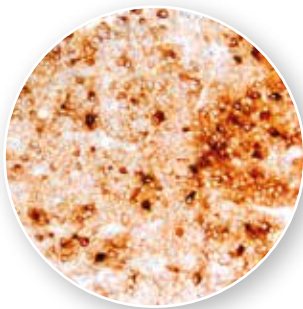


# Immunohistology

## PD-L1



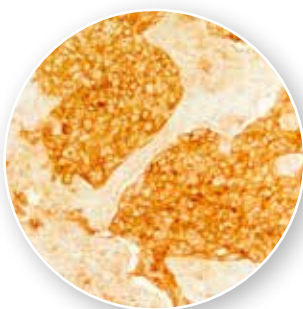
## CAL10: a monoclonal rabbit antibody to PD-L1 (Programmed Cell Death Ligand 1)



PD-L1 immunostain of NSCLC (NordiQC assessment 2017, Run C1x – PD-L1)



PD-L1 immunostain of control cells (NordiQC assessment 2017, Run C1x – PD-L1)



PD-L1 immunostain of NSCLC (ESP Lung EQA 2017)

Recent clinical trials demonstrate the efficacy of checkpoint-targeted therapy using PD1 or PD-L1 antibodies in various cancers. PD-L1 expression has been shown to be a potential predictive biomarker for selecting responders to anti-PD-1/PD-L1 antibody treatment. Immunohistochemistry with antibodies included in so-called companion diagnostics kits or antibodies validated in-house is currently used for the detection of PD-L1 expression. The available assays use different PD-L1 clones recognizing various epitopes of PD-L1 with different staining protocols on different platforms for a single biomarker. This situation results in practical and financial challenges for pathology laboratories [1,2].

**In most countries pathologists are free to choose their preferred method for the detection of PD-L1 expression** if the method is validated and ro-

ust enough to minimize false positive and false negative staining. Harmonizing studies are underway in order to improve standardization and assist pathologists in the clinical decision-making [3]. One goal of these studies is to make all approved anti-PD-L1/PD1 therapies available to patients irrespective of the chosen PD-L1 assay.

Next to the trial-validated assays, different PD-L1 antibodies not associated with a kit are available on the market [4]. In comparison to companion diagnostic kits the use of these „free“ antibodies in laboratory-developed tests (LDT) reduces the costs significantly.

Zytomed Systems offers a CE/IVD classified rabbit monoclonal anti-PD-L1 antibody which has been successfully used in ring trials by various pathological laboratories.

### ► CAL10 in NSCLC

PD-L1 immunohistochemistry was conducted on the IntelliPATH FLX™ for the external quality assessment (EQA) „PD-L1 (IHC) in NSCLC“ of QuIP, the relevant quality assurance initiative for pathology in Germany. Successful participation was achieved by using the following protocol:

**Dewaxing/Pre-treatment:** DC-Modul (Biocare Medical), OmniPrep Solution pH 9,0 (Zytomed Systems)

**PD-L1 antibody:** Clone CAL10, Dilution 1:100 in Antibody diluent (Zytomed Systems)

**Detektion:** 2-step polymer kit and DAB (Zytomed Systems)

**Scoring:**  
Proportion of positive tumor cells < 1% = 0  
Proportion of positive tumor cells 1-49% = 1  
Proportion of positive tumor cells > 50% = 2

### ► CAL10 in breast carcinoma

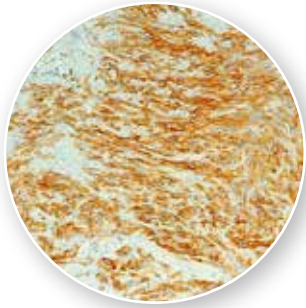
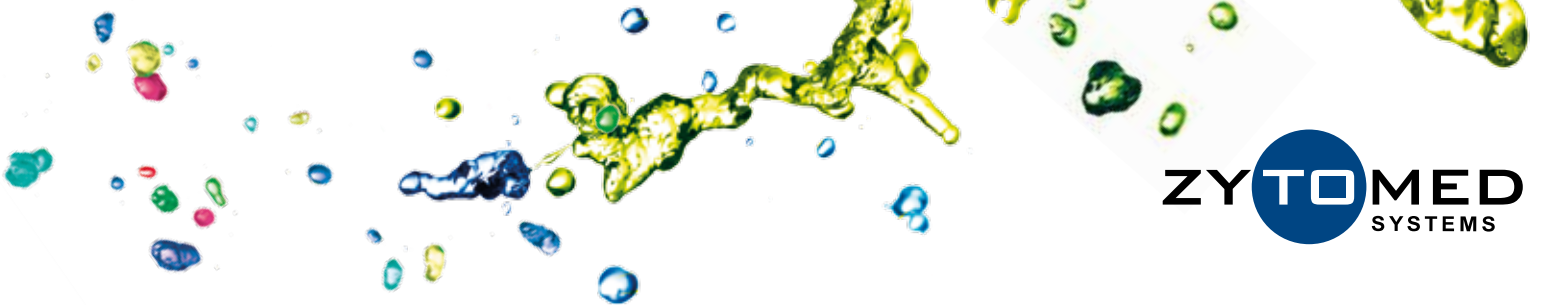
Tejashree Karnik and colleagues from the University of Kansas Medical Center presented the results of their comparative study at the USCAP Meeting in San Antonio, Texas in March 2017. 136 breast carcinomas including primary cancer and metastatic diseases were stained with PD-L1 clones 22C3 and SP263 of companion kits and clone CAL10 which is available as a „free“ antibody. The three antibodies performed equally well and exhibited equal if not identical results [5].

The authors concluded:

*Thus, in our view, as in the case with quantitation of PD-L1 in lung cancer and melanoma, pathologists have the option of utilizing less expensive reagents for the evaluation of this marker in breast cancer.*

# Immunohistology

## PD-L1



PD-L1 immunostain of malignant melanoma (QuIP EQA 2017)

### ► CAL10 in malignant melanoma

PD-L1 immunohistochemistry on a half automated immunostainer was conducted for the EQA „PD-L1 (IHC) in malignant melanoma“ of QuIP. The excellent result of 20 of 20 possible points were achieved using the following protocol:

Dewaxing:	Xylene and decreasing ethanol series
Pre-treatment:	Steamer, 35 min/95 °C, HIER Citrate Buffer pH6.0 (Zytomed Systems)
PD-L1 antibody:	Clone CAL10, Dilution 1:100 in Antibody diluent (Zytomed Systems)
Detection:	HRP-Streptavidin-Biotin-system and DAB (Zytomed Systems)

Scoring: Proportion of positive tumor cells < 5% = 1  
Proportion of positive tumor cells > 5% = 2

### ► Gene information and terms

Gene	Description	Synonyme	Gene ID	Gene localization
<b>PDCD1</b>	Programmed Cell Death 1	CD279, hSLE1, PD-1, PD1, SLEB2	MIM600244	2q37.3
<b>CD274</b>	CD274 Molecule	B7-H, B7-H1, B7H1, PD-L1, PDL1, PDCD1LG1	MIM605402	9p24.1
<b>PDCD1LG2</b>	Programmed Cell Death 1 Ligand 2	B7-DC, bA574F11.2, Btdc, CD273, PD-L2, PDL2	MIM605723	9p24.1

### ► Antibodies against PD-L1 and PD-L2

Description	Format	Dilution	Amount	Cat. No.
<b>PD-L1 (CD274)</b> Clone: CAL10 Host: Rabbit	Ready-to-use	-	6 ml	RBG063
	Concentrate	1:100 – 1:200	0.5 ml	RBK063-05
<b>PD-L2 (CD273)</b> Clone: polyclonal Host: Rabbit	Concentrate	ca. 1:400	100 µg (0.1 ml)	603-2393
<b>PD1 (CD279)</b> Clone: polyclonal Host: Rabbit	Ready-to-use	-	7 ml	516-18661
	Concentrate	1:100	0.5 ml	516-18662
			1 ml	516-18664

### ► Controls for PD-L1 expression

Description	Amount	Cat. No.
<b>CD274 (PD-L1) Expression IHC Reference Standard</b> Paraffin sections of 4 cell line cores expressing different level of PD-L1 (-/+ /++ /+++)	1 pack includes 5 slides	HD787

### ► Literature

- [1] Kerr KM, Nicolson MC. Non-Small Cell Lung Cancer, PD-L1, and the Pathologist. Arch Pathol Lab Med 140:249-254, 2016
- [2] Kerr KM, Hirsch FR. Programmed Death Ligand-1 Immunohistochemistry: Friend or Foe? Arch Pathol Lab Med 140:325-331, 2016
- [3] Scheel AH *et al.* Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas. Mod Pathol 29:1165-1172, 2016
- [4] Hutarew G. PD-L1 testing, fit for routine evaluation? From a pathologist's point of view. memo 9:201-206, 2016
- [5] Karnik T *et al.* PD-L1 in Breast Cancer: Comparative Analysis of Three Different Antibodies (Poster Session II/13, USCAP-Meeting März 2017, San Antonio, Texas)



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Immunohistology