CE

Monoclonal Mouse Anti-Human PD-L1

Clone 22C3

Code M3653

Intended use

For in vitro diagnostic use.
Monoclonal Mouse Anti-Human PD-L1, Clone 22C3, is intended for use in immunohistochemistry. This antibody labels PD-L1 in normal and neoplastic tissue. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a certified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

Synonyms for antigen

Programmed Death-Ligand 1 *(1).*

Summary and explanation

Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T cell proliferation and cytokine production (2). Up-regulation of PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors (3). Blockade of PD-1 interaction with PD-L1 and PD-L2 releases PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response (4).

Refer to our *General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures.

Reagent provided

Monoclonal mouse antibody provided in liquid form in 0.05 mol/L Tris-HCl, 0.015 mol/L sodium azide, 1% bovine serum albumin, pH 7.2.

Clone: 22C3 (5). Isotype: IgG1.
Mouse IgG concentration mg/L: See label on vial.

The protein concentration between lots may vary without influencing the optimal dilution. The titer of each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

Immunogen

Human extracellular domain of PD-L1 (Phe19-Thr239) fused to a human IgG1 fragment (R&D Systems Catalogue No. 156-B7-100)

Specificity

In Western blotting of recombinant human PD-L1 protein, Monoclonal Mouse Anti-Human PD-L1, Clone 22C3 labels a band corresponding to ~40 kDa.

Precautions

1. For professional users.
2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.
4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
5. Unused solution should be disposed of according to local, State and Federal regulations.

Storage

Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Agilent Pathology Support.

Specimen preparation

Paraffin sections: The antibody can be used for labeling paraffin-embedded tissue sections fixed in formalin. Tissue specimens should be cut into sections of approximately 4 µm.

Pre-treatment: Please refer to EnVision™ FLEX Target Retrieval Solution, Low pH (50x) (Code K8005) for instructions on how to perform pretreatment of slides.

Staining procedure

These are guidelines only. Optimal conditions may vary depending on specimen type and preparation method, and should be validated individually by each laboratory. The performance of this antibody should be established by the user when utilized with other manual staining systems or automated platforms.

Dilution: The recommended dilution of Monoclonal Mouse Anti-Human PD-L1, Clone 22C3, Code M3653, is 1:50. Dilute the antibody in Dako Antibody Diluent with Background-Reducing Components (Code S3022). Incubate pretreated tissue sections for 30 minutes at room temperature.

Negative control: The recommended negative control reagent is Dako Negative Control, Mouse IgG1 (Code X0931) diluted to the same IgG1 concentration as the primary antibody. Unless the stability of the diluted antibody and negative control has been established in the actual staining procedure, dilute these reagents immediately prior to use. Positive and negative controls should be run simultaneously with patient specimens.

Visualization: The recommended EnVision™ FLEX HRP visualization system is using 30-minute incubation at room temperature. The incubation time for EnVision™ FLEX+ Mouse (LINKER) (Code K8021/K8022) is 30 minutes at room temperature. Follow the procedure enclosed with the selected visualization system(s).

Note: Use EnVision™ FLEX Target Retrieval Solution, **Low pH** (50x) (Code K8005) for HIER.

The incubation time for DAB enhancer (S196131-2) is 5 minutes at room temperature.

Counterstaining: The recommended counterstain is EnVision™ FLEX Hematoxylin (Code K8008/K8018). For optimal results, non-aqueous, permanent mounting medium is recommended.

Controls: Positive and negative control tissues should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include tonsil and the cells/structures should display reaction patterns as described for this tissue in the "Performance characteristics" section.

Staining interpretation

The cellular staining pattern is membranous.

Performance characteristics

Normal tissues:

Plasma membrane staining was observed on immune cells and cells of epithelial origin. Cytoplasmic staining was noted in some cell types but was not recorded as positive staining. Table 1 summarizes PD-L1 immunoreactivity on the recommended panel of normal tissues. All tissues were formalin-fixed and paraffin-embedded and stained with Monoclonal Mouse Anti-PD-L1, Clone 22C3 according to the instructions in this package insert. There were no unexpected results observed in cell types or tissue types tested. The observed staining was consistent with the reported literature for PD-L1 IHC expression in normal tissues (1, 2).

Table 1: Summary of Monoclonal Mouse Anti-PD-L1, Clone 22C3 normal tissue reactivity

Tissue Type (# tested)	Positive Tissue Elements	Tissue Type (# tested)	Positive Tissue Elements
Adrenal (3)	0/3	Parathyroid (3)	1/3 Glandular epithelium
Bone marrow (3)	3/3 Megakaryocyte		
Breast (3)	0/3	Pancreas (3)	0/3
Cerebellum (3)	0/3	Pituitary (3)	1/3 Anterior hypophysis
Cerebrum (3)	0/3		1/3 Posterior hypophysis
Cervix (3)	1/3 epithelial cells	Prostate (2)	2/2 epithelium
Colon (3)	1/3 Lymphocytes	Salivary Gland (3)	0/3
	1/3 Macrophages		
Esophagus (3)	0/3	Skin (3)	0/3
Kidney (3)	1/3 Tubular epithelium	Small Intestine (3)	0/3
Liver (3)	1/3 Macrophages	Spleen (3)	2/3 Macrophages
	1/3 Hepatocytes		
Lung (3)	3/3 Macrophages	Stomach (3)	2/3 Lymphocytes
			1/3 gastric glands
Mesothelial cells (2)	0/2	Testis (3)	0/3
Muscle, cardiac (3)	0/3	Thymus (3)	3/3 Medulla
Muscle, skeletal (3)	0/3	Thyroid (3)	0/3
Nerve, peripheral (3)	0/3	Tonsil (3)	3/3 Crypt epithelium
			2/3 macrophages
Ovary (3)	0/3	Uterus (3)	0/3

Abnormal tissues:

Plasma membrane staining was observed on immune cells and cells of epithelial origin. Cytoplasmic staining was noted in some cell types but was not recorded as positive staining. Table 2 summarizes PD-L1 immunoreactivity on a panel of neoplastic tissues. All tissues were formalin-fixed and paraffin-embedded and stained with Monoclonal Mouse Anti-PD-L1, Clone 22C3 according to the instructions in this package insert. There were no unexpected results observed in the tumor specimens tested. The

observed staining was consistent with the reported literature for PD-L1 IHC expression in neoplastic tissues (1-4).

Table 2: Summary of Monoclonal Mouse Anti-PD-L1, Clone 22C3 neoplastic tissue reactivity

Tumor Type	Location	PD-L1 positive/ N=159
Adenocarcinoma	Appendix	0/1
	Breast, DCIS	0/2
	Breast, invasive ductal	0/7
	Breast, invasive ductal metastatic to lymph node	0/1
	Cervix, endocervical type	0/1
	Colon	0/5
	Colon, metastatic to liver	0/1
	Colon, mucinous	0/1
	Esophagus	0/1
	Gallbladder	1/5
	GI, metastatic to lung	0/1
	Head & neck, hard palate	0/1
	Lung	1/4
	Ovary	0/1
	Ovary, endometrioid	0/1
	Ovary, mucinous	0/1
	Ovary, serous	0/1
	Pancreas	0/2
	Pancreas, ductal	0/3
	Prostate	0/5
	Rectum	0/4
	Salivary/parotid gland	0/2
	Small intestine	0/2
	Stomach	0/6
	Stomach, mucinous	0/1
	Thyroid, follicular	0/1
	Thyroid, follicular-papillary	0/1
	Thyroid, papillary	0/3
	Uterus, clear cell	0/1
	Uterus, endometrium	0/3
Adrenocortical carcinoma	Adrenal	0/1
Astrocytoma	Cerebrum	0/3
Basal cell carcinoma	Skin	0/1
Carcinoma	Nasopharyngeal, NPC	0/1
Chondrosarcoma	Bone	0/1
Chordoma	Pelvic cavity	0/1
Embryonal carcinoma	Testis	0/1
Ependymoma	Brain	0/1
Glioblastoma	Brain	0/1
Hepatoblastoma	Liver	0/1
Hepatocellular carcinoma	Liver	0/5
Islet Cell tumor	Pancreas	0/1
Interstitialoma	Colon	0/1
	Rectum	0/1
	Small intestine	0/1
Leiomyosarcoma	Soft tissue, chest wall	0/1
	Bladder	0/1

Lymphoma		
Anaplastic Large Cell	Lymph node	0/1
Diffuse B-cell	Lymph node	0/4
Hodgkin	Lymph node	2/2
Non-Hodgkin	Lymph node	1/1
Medulloblastoma	Brain	0/1
Medullary carcinoma	Thyroid	0/1
Melanoma	Rectum	0/1
	Nasal cavity	0/1
Meningioma	Brain	0/2
Mesothelioma	Peritoneum	0/1
Neuroblastoma	Retroperitoneum	0/1
Neurofibroma	Soft tissue, lower back	0/1
Osteosarcoma	Bone	0/2
Pheochromocytoma	Adrenal	0/1
Primitive Neuroectodermal Tumor (PNET)	Retroperitoneum	0/1
Renal Cell carcinoma		
Papillary	Kidney	0/1
Clear Cell	Kidney	0/6
Rhabdomyosarcoma	Soft tissue, embryonal	0/1
	Prostate	0/1
	Retroperitoneum	0/1
Seminoma	Testis	0/2
Signet Ring Cell carcinoma	Metastatic colon signet ring cell carcinoma to ovary	0/1
	Colon	0/1
Small cell carcinoma	Lung	0/1
Spermatocytoma	Testis	0/2
Squamous Cell carcinoma	Metastatic esophageal squamous cell carcinoma to lymph node	0/1
	Cervix	2/5
	Esophagus	0/7
	Head & neck	0/2
	Lung	1/2
	Skin	0/2
	Uterus	0/1
Synovial Sarcoma	Pelvic cavity	0/1
Thymoma	Mediastinum	1/1
Transitional Cell carcinoma	Bladder	0/6
	Kidney	0/1

References

1. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. International Immunol 2007(19); 7:813.
2. Brown JA, Dorfman DM, Ma F-R, Sullivan EL, Munoz O, Wood CR, et al. Blockade of Programmed Death-1 Ligands on Dendritic Cells Enhances T Cell Activation and Cytokine Production. J Immunol 2003;170:1257.
3. Cooper WA, Tran T, Vilain RE, Madore J, Seliger CI, Kohonen-Cornish M, et al. PD-L1 expression is a favorable prognostic factor in early stage non-small cell carcinoma. Lung Cancer 2015; 89:181.
4. Chen B, Chapuy B, Ouyang J et al. PD-L1 Expression Is Characteristic of a Subset of Aggressive B-cell Lymphomas and Virus-Associated Malignancies. Clin Cancer Res 2013; 19:3462-3473.

Explanation of symbols

REF	Catalogue number		Temperature limitation	IVD	In vitro diagnostic medical device
	Manufacturer	LOT	Batch code		Contains sufficient for <n> tests
	Use by		Consult instructions for use	EC REP	Authorized representative in the European Community

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