CE/ IVD

PD-L1 (Clone QR1) Rabbit Monoclonal Antibody

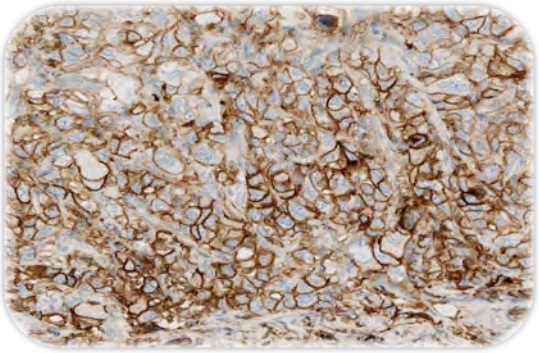

Figure 1 Metastatic NSCLC stained with PD-L1 (QR1)

Product identification

1-PR292-02	100 µl Concentrate
1-PR292-05	500 µl Concentrate
1-PR292-07	1 ml Concentrate
2-PR292-07	1 ml Ready-to-use
2-PR292-10	3 ml Ready-to-use
2-PR292-13	7 ml Ready-to-use

Further packing sizes possible.

Intended use

For *in vitro* diagnostic use.
PD-L1 (QR1) is a rabbit monoclonal anti-human antibody for immunohistochemical use. The primary antibody is intended for qualitative detection of antigens in formalin-fixed, paraffin-embedded (FFPE) tissue sections. The antibody may be used manually or with autostainer. Authorized and skilled personnel may only use the product. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic laboratory test results. A qualified pathologist must perform evaluation.

Summary and explanation

Programmed Death Ligand 1 (PD-L1), also known as CD274 and B7-H1, is a transmembrane protein expressed on the surface of resting T-cells.
Binding to its receptor PD-1, a T-cell immune checkpoint, T-cell activation is inhibited and autoimmune reaction is stopped. Some tumor cells use this mechanism to prevent apoptosis and obtain resistance against CD8+ T-cell mediated cell lysis.
PD-L1 (QR1) is suitable to detect sentinel lymph node melanoma, renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC) as well as triple-negative breast cancer (TNBC).
The antibody produces membranous and/or cytoplasmic staining.

Principle of the procedure

The stated primary antibody is suitable for immunohistochemical staining of FFPE tissue sections based on specific antigen-antibody reaction. Using a detection system linked to HRP or alkaline phosphatase the antigen visualization is performed via specific binding of the primary antibody. Secondary antibody is binding to the primary antibody, and this complex labeled by the enzyme complex. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. Each step is incubated for a precise time and temperature and requires interposed washing steps. The specimen may then be counterstained. Results are interpreted using a light microscope.

Materials provided

Primary antibody	PD-L1 (QR1)
Host	Rabbit
Subclass	IgG1
Immunogen	Synthetic peptide derived from a region of PD-L1 protein
Antibody concentrate	Concentrated antibody in TRIS (pH 7.4) with < 0.1 % sodium azide
Recommended working dilution range	1:100 – 1:200
Ready-to-use antibody	Prediluted antibody in TRIS (pH 7.4) with < 0.1 % sodium azide

Product label shows the specific lot number.

Prediluted antibody is ready-to-use and optimized for staining. No further dilution, reconstitution, mixing, or titration is needed.
Antibody concentrate is optimized for dilution within dilution range using ProTaqs® Antibody Diluent for IHC (Cat. No. 400100295). Indicated dilution range should be considered as recommendation and depends on different facts (tissue, fixation, incubation conditions, etc.). Optimum titration to be determined in user's own system.

Materials required but not provided

The following materials may be required for staining but are not provided with the primary antibody.
- Positive and negative controls
- Microscope slides (positively charged) and cover slips
- Water bath, e.g. Tissue Float Bath (Cat. No. 990100720)
- Humidified chamber, e.g. Slide Humidore (Cat. No. 990100200)
- Staining jars
- Timer
- Xylene or xylene alternative, e.g. ProTaqs® Clear (Cat. No. 400301105)
- Ethanol
- Deionized or distilled water
- Antibody diluent, e.g. ProTaqs® Antibody Diluent for IHC (Cat. No. 400100295)
- Antigen retrieval reagent, e.g. ProTaqs® Antigen Enhancer IV (Cat. No. 401602392)
- Detection system, e.g. ProTaqs® Essencial with AEC (e.g. Cat. No. 300120300) or ProTaqs® Essencial with DAB (e.g. Cat. No. 300120200)
- Wash buffer: TBS (Cat. No. 402000192) or TBS-Tween20 (Cat. No. 402000492)
- Tap water/bluing reagent (e.g. ammonia water)
- Light microscope

Storage and handling

Store at 2 – 8 °C.

When stored correctly antibody is stable to the expiration date indicated on the vial. Do not use after expiration date.
To ensure proper reagent delivery and stability of the antibody, replace the dispenser cap after every use and immediately place the bottle cool in an upright position.

Specimen preparation

Routinely processed, FFPE tissues are suitable for use with this primary antibody when used with ProTaqs® detection kits (see section "Materials required but not provided"). The recommended tissue fixative is 10 % neutral buffered formalin. Variable results may occur as a result of prolonged fixation or special processes such as decalcification of bone marrow preparations. Thickness of tissue sections should be 2 – 5 µm. Slides should be stained as soon as possible, as antigenicity of cut tissue sections may diminish over time.
It is recommended to stain positive and negative controls simultaneously with unknown specimens.
The optimum pretreatment protocol must be determined in user's own system.

Warnings and precautions

1. Application only by qualified and trained personnel.
2. There are no estimated health risks, if the product is used as directed. MSDS is available on request.
3. Product contains sodium azide as preservative. Pure sodium azide is toxic. The concentration of sodium azide in this reagent is < 0.1 % and is not classified hazardous. See MSDS.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Do not use reagents after expiration date.
6. Take reasonable precautions when handling reagents. Use protective clothing and gloves.
7. All hazardous materials should be disposed according to guidelines for hazardous waste disposal. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
8. Avoid microbial contamination of reagents as it may cause incorrect results.

Staining procedure

Primary antibody has been optimized for use in combination with ProTaqs® detection kits. The following data are recommendations. Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to adjust incubation times. The optimum protocol must be determined in user's own system.

Antigen retrieval: HIER; Boil tissue sections in ProTaqs® Antigen Enhancer IV for 30 min followed by cooling at room temperature (RT) for 30 min.

Incubation of primary antibody for 90 min at RT.

Staining protocol: Follow the procedure described in the instructions of the used detection system.

Recommendations for staining protocols:
1. VENTANA BenchMark Ultra

Dilution	– 1:100
Pretreatment	– CC1 64 min
Incubation	– 32 min at RT
Detection	– UltraView + Amplification

2. Leica Bond

Dilution	– 1:100
Pretreatment	– HIER: ER2 30 min
Incubation	– 60 min
Detection	– Bond Polymer Refine

3. Dako Autostainer

Dilution	– 1:100
Pretreatment	– Citrate (pH 6.0) 20 min, PT module
Incubation	– 30 min at RT
Detection	– Dako EnVision

4. Manual

Dilution	– 1:100
Pretreatment	– TRIS-EDTA-citrate (pH 7.8) at 100 °C for 25 min
Incubation	– 60 min at RT

Quality control procedures

Positive tissue control
A positive tissue control must be run with every staining procedure performed for monitoring the correct performance of processed tissues and test reagents.
If the positive tissue controls fail to demonstrate appropriate positive staining, results with the test specimens must be considered invalid.

Examples for positive tissue control:
Human placental tissue shows moderate to strong uniform staining of the membrane and cytoplasm of trophoblast-lineage cells.

Negative tissue control
Negative tissue controls provide an indication of non-specific background staining. If specific staining occurs in the negative tissue control sites, results with the patient specimens must be considered invalid.

The variety of cell types present in most tissue sections offers internal negative control sites. Therefore, the same tissue used for the positive tissue control may be used as the negative tissue control.
Examples for negative tissue control:
Placental stromal tissue and vasculature.

Discrepancies
If quality control results do not meet specifications, patient results are invalid. Identify and correct the problem, then repeat the entire procedure with the patient samples.

Negatives control reagent
A negative control reagent is used in place of the primary antibody to evaluate nonspecific staining. Host species and incubation time should be similar to primary antibody.

Interpretation of results

The immunostaining procedure causes a colored reaction product to precipitate at the antigen sites localized by the primary antibody.
PD-L1 (QR1) provides membranous and/or cytoplasmic staining of tumor cells. Immune cells demonstrate linear membrane, diffuse cytoplasmic, and/or punctate staining.

A qualified pathologist experienced in immunohistochemistry procedures must evaluate positive and negative tissue controls before interpreting patient specimens.
Positive staining intensity should be assessed within the context of any background staining of the negative reagent control.
Note: A negative result means that the antigen in question was not detected, not that the antigen is absent in the cells or tissue assayed. A panel of antibodies may be used to verify the results. Additionally, the morphology of each tissue sample should be examined utilizing a hematoxylin and eosin stained section. A qualified pathologist must interpret the patient's morphologic findings and pertinent clinical data.

Performance characteristics

Table 1 Testing of normal FFPE tissue sections

Tissue	***Positive/total cases***
Placenta	3/3
Tonsil	2/2
Lymph node	1/1
Prostate	1/1
Kidney	1/1

Table 2 Testing of neoplastic FFPE tissue sections

Tissue	*Positive/total cases*
Non-small cell lung cancer (NSCLC)	2/2
Low differentiated pulmonary adenocarcinoma	1/1

Troubleshooting

1. This reagent is “for professional use only” as immunohistochemistry is a multiple step process that requires specialized training in the selection of the appropriate reagents, tissues, fixation and processing, preparation of the immunohistochemistry slide, choice of detection system, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or incorrect results.
3. Only intact cells should be used for interpretation of staining results, as degenerated cells show non-specific staining.
4. If no staining is occurs, control application order of reagents. Follow all indications given in the instructions for use.
5. Do not allow the sections to dry out.
6. If weak staining occurs, pay attention during staining steps to freshly prepared chromogen, incubation times and temperatures, as well as accurate draining off of reagents.
7. Avoid surplus background staining by optimal removal of paraffin, washing of slides and dilution of primary antibody. If excessive background staining occurs, high levels of endogenous biotin may be present (unless a biotin-free detection system is being used). A biotin blocking step should be included.
8. Excessive or incomplete counterstaining may compromise proper interpretation of results.
9. Sodium azide inactivates HRP, which may lead to false results. Wash sections in sodium azide free buffer.
10. Prediluted antibodies are ready-to-use and optimized for staining. Further dilution may lead to incorrect results.
11. After successful validation users may dilute antibody concentrates according to requirements. Appropriate controls must be employed and documented.
12. Contact quartett customer service in case of any uncertainties.

Limitations

Errors excepted.
For *in vitro* diagnostic use. For laboratory use only.
This data sheet contains general information. Optimum performance requires appropriate specimen handling, preparation, and storage as described. The performance of the product was established using the procedures provided in this package insert only and modifications to these procedures may lead to changes in efficiency. Non-application as prescribed in this data sheet leads to loss of all liability. Optimal performance requires adequate specimen quality as well as appropriate sample preparation. Application in combination with diagnostic devices requires prior validation. Any changes in product, composition, implementation, as well as use in combination with any reagents other than recommended herein is not allowed; users are responsible themselves for those changes and have to perform prior validation. Manufacturer is not liable for incorrect results and events resulting thereof, as well as for incorrect results due to visual evaluation.
Authorized and skilled personnel may only use the product. The clinical interpretation of any test results should be evaluated within the context of the patient’s medical history and other diagnostic laboratory test results. A qualified pathologist must perform evaluation. We do not take responsibility for any possible damage including personal injury, time or effort on economic loss caused by this product. Our warranty is limited to the price paid for the product.

Literature

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cert. by TÜV Rheinland Group EN ISO 13485:2016 & ISO 9001:2015

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Explanation of symbols

REF	Bestellnummer Catalog number		Verwendbar bis Use by
LOT	Chargenbezeichnung Batch code		Temperaturbegrenzung Temperature limitation
IVD	In Vitro Diagnostika In vitro diagnostic agent		Bei beschädigter Verpackung nicht verwenden Do not use if package damaged