

Rabbit monoclonal antibody against p16 (QR019) *In Vitro Diagnostic Use (IVD)*

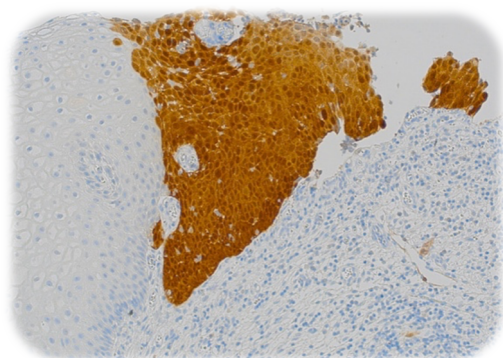


Figure 1 Cone CIN III stained with anti-p16 (QR019)

Product identification

C-P010-025	25 µl	Concentrate
C-P010-01	0.1 ml	Concentrate
C-P010-05	0.5 ml	Concentrate
C-P010-10	1 ml	Concentrate
P-P010-30	3 ml	Ready-to-use
P-P010-70	7 ml	Ready-to-use
P-P010-150	15 ml	Ready-to-use

Intended use

Anti-human antibody for *in vitro* diagnostic use. The primary antibody is intended for the qualitative detection of associated antigens as listed in the section 'Summary and explanation'. It is intended to be used within an immunohistochemistry (IHC) procedure on formalin-fixed, paraffin-embedded (FFPE) tissue sections followed by light microscopy visualization to aid tumor diagnosis. The antibody may be used manually or with any automated staining platform.

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

p16, also designated p16(INK4a), cyclin-dependent kinase inhibitor 2A or CDKN2A, is a tumor suppressor protein that slows cell division by slowing the progression of the cell cycle from the G1 phase to the S phase. Mutations in the p16 gene - associated with loss or overexpression of the protein - are associated with increased risk of a wide range of cancers and cancer precursor lesions. When overexpressed, the protein is detected both in the nucleus and cytoplasm.

p16 is expressed in almost 100% of cervical carcinomas, especially in squamous cell carcinomas of the cervix^[1-2]. The vast majority of these carcinomas are associated with human papilloma virus (HPV) infection, especially high-risk HPV types such as HPV 16 and 18. The overexpression of p16 is caused by the inactivation of the retinoblastoma protein (Rb) by the viral E7 oncogene, which leads to the upregulation of p16. Strong and full thickness staining of p16 in the cervix epithelium is highly supportive of high grade squamous epithelial lesion (HSIL), while weak and intermediate staining favors low grade squamous epithelial lesion (LSIL).

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 horseradish peroxidase (HRP) or alkaline phosphatase (AP) the antigen visualization is performed via specific binding of the primary antibody. Secondary antibody is binding to the primary antibody, and the enzyme complex labels this 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	Anti-p16 (QR019)
Host	Rabbit
Subclass	IgG
Immunogen	Synthetic peptide of human p16
Antibody concentrate	Concentrated antibody in TRIS (pH 7.4) with < 1 % sera (bovine, donkey) and < 0.1 % sodium azide
Recommended working dilution range	1:100 – 1:200
Ready-to-use antibody	Prediluted antibody in TRIS (pH 7.4) with < 1 % sera (bovine, donkey) and < 0.1 % sodium azide

Product label shows the specific lot number. Each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

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 Q Diluent for IHC (Cat. No. AD-001-xxxx). Indicated dilution range should be considered as recommendation and depends on different facts (tissue, fixation, incubation conditions, etc.). Optimum dilution to be determined in user's own system.

Materials required but not provided

- Positive and negative controls
- Microscope slides (positively charged) and cover slips
- Staining jars
- Timer
- Xylene or xylene alternative, e.g. Q Dewax Solution (Cat. No. DW-001-xxxx)
- Ethanol
- Deionized or distilled water
- Heating equipment for tissue pretreatment step
- Antibody diluent, e.g. Q Diluent for IHC (Cat. No. AD-001-xxxx)
- Antigen retrieval reagent, e.g. Q Retrieval Low pH 6.0 (Cat. No. AR-001-0120) or Q Retrieval High pH 9.0 (Cat. No. AR-002-0120)
- Detection system, e.g. PolyQ Stain kits and appropriate chromogen
- Wash buffer, e.g. TBS (Cat. No. BU-006-xxxx) or TBS-Tween20 (Cat. No. BU-007-xxxx)
- Blocking reagent
- Hematoxylin
- Mounting medium
- Light microscope

Storage and handling

Store at 2 – 8 °C.

When stored correctly, the antibody is stable up to the expiration date indicated on the vial. This also applies to the shelf life after opening or after dilution of the concentrate by the end user. 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. 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, which should be placed on positively charged slides, should be 2 – 5 µm. Pretreatment of deparaffinized tissue with heat-induced epitope retrieval (HIER) is recommended. Slides should be stained as soon as possible, as antigenicity of cut tissue sections may diminish over time.

The optimum pretreatment protocol must be determined in user's own system.

Warnings and precautions

1. Authorized and skilled personnel may only use the product.
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 % which is not classified as hazardous.
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 PolyQ Stain detection systems. 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 Q Retrieval for approximately 20 min followed by cooling at room temperature (RT) for approximately 20 min.

Incubation of primary antibody for 30 – 60 min at RT.

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

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. Known positive tissue controls should not be utilized as an aid in determining a specific diagnosis of patient sample.

If the positive tissue controls fail to demonstrate appropriate positive staining, results with the test specimens must be considered invalid.

Example for positive tissue control:

- Tonsil (Moderate to strong nuclear and cytoplasmic staining reaction in scattered reticulated crypt epithelial cells, weak but distinct nuclear and cytoplasmic staining reaction in dispersed germinal center macrophages/dendritic cells)
- Pancreas (At least moderate staining in islet of Langerhans)

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.

Example for internal negative tissue control:

- Tonsil (No staining reaction should be seen in the vast majority of lymphocytes and normal superficial squamous epithelial cells)
- Pancreas (No staining in acinar cells)
- Normal cervix uteri (No staining in epithelial cells)

Discrepancies

If quality control results do not meet specifications, patient results are invalid. Identify and correct the problem (see section "Troubleshooting"), then repeat the entire procedure with the patient samples.

Negative control reagent

A negative control reagent is used in place of the primary antibody to evaluate non-specific 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.

Cellular localization: Nuclear, cytoplasmic. Positive staining is defined as "block" staining: strong nuclear and cytoplasmic expression in a continuous segment of cells. Cytoplasmic only staining, diffuse blush / weak intensity staining and other focal / patchy patterns should be considered negative.

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

The antibody has been validated by IHC using FFPE human tissue sections of different types of healthy and neoplastic tissues.

Table 1 Testing of healthy FFPE tissue sections

Tissue	Positive/total cases
Tonsil	12/12
Pancreas	10/10

Table 2 Testing of neoplastic FFPE tissue sections

Tissue	Positive/total cases
Cervix squamous cell carcinoma	29/29
Cervix adenocarcinoma	10/10
Uterus carcinoma	12/12

Analytical performance

The antibody passed all analytical performance tests. Analytical sensitivity has been determined by measuring concordances with known positive tissues, and analytical specificity has been determined by measuring concordances with known negative tissues with at least 90% overall concordance between the new test and the expected results each. Results are 100% each. Trueness has been verified by comparison with an independent product, and is confirmed as the results match 100%.

The method has a high level of precision - repeatability within run (carried out several times on the same day by the same analyst with the same instrument), reproducibility between run (carried out on different days by different analysts with different instruments) and reproducibility from lot to lot (results of a new reagent lot compared to those of a previously used lot) are confirmed with 100% each. Trueness and precision result in a high level of accuracy of measurement for the method.

Since IHC is a qualitative detection method that provides information on whether the corresponding antigen is present or not, the concentration-related parameters detection and quantification limits, cutoff/tolerance limit, measuring range and linearity are not applicable and are all unable to be defined for this product.

Clinical performance

For the assessment of clinical performance, authorized staining pictures provided by customers using clone QR019 have been evaluated to prove clinical evidence with the aim to achieve a robust and correct visualization of the target antigen in clinical samples with unknown expression levels, thereby contributing to a valid diagnosis.

Clinical performance parameters have been calculated with a total of 29 cervical squamous cell carcinomas, 10 cervical adenocarcinomas, 12 uterine carcinomas^[own data] as well as 10 tonsil and 10 pancreas tissues^[own data].

Summarized diagnostic sensitivity, diagnostic specificity, PPV, NPV and LR- resulted in 100%, 100%, 100%, 100% and 0 (interpreted as convincing diagnostic evidence), respectively. Results of clinical performance evaluation are in accordance with scientific peer-reviewed literature as shown in section 'Summary and explanation'.

Limitations

1. For *in vitro* diagnostic use.
2. For laboratory use only.
3. 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.
4. Tissue staining is dependent on the handling, processing and storage 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. Optimal performance requires adequate specimen quality as well as appropriate sample preparation.

5. Excessive or incomplete counterstaining may compromise proper interpretation of results.
6. False positive results may be seen because of non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudo peroxidase activity (erythrocytes), endogenous biotin (example: liver, brain, kidney) or endogenous peroxidase activity (cytochrome C).
7. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results because of the effect of autoantibodies or natural antibodies.
8. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen may exhibit nonspecific staining with HRP.
9. Unexpected results may occur due to biological variability of antigen expression in neoplasms or other pathological tissues.
10. 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. Staining must be performed in a certified, licensed laboratory under the supervision of a qualified pathologist who is responsible for evaluation and assuring the adequacy of positive and negative controls. Manufacturer is not liable for incorrect results due to visual evaluation.
11. Prediluted antibodies are ready-to-use and optimized for staining. Further dilution may lead to incorrect results.
12. After successful validation users may dilute antibody concentrates according to requirements. Appropriate controls must be employed and documented.
13. 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. 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.
14. Application in combination with diagnostic devices, e.g. an automated staining platform, requires prior validation before staining patient specimen.
15. 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.

Troubleshooting

1. Only intact cells should be used for interpretation of staining results, as degenerated cells show non-specific staining.
2. If no staining occurs, control application order of reagents. Follow all indications given in the instructions for use.
3. Do not allow the sections to dry out.
4. 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.
5. 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.

6. Sodium azide inactivates HRP, which may lead to false results. Wash sections in sodium azide free buffer.
7. Contact quartett customer service in case of any uncertainties.

Literature

[1] Sano T, Oyama T, Kashiwabara K et al. (1998). Pathol Int. 48(8):580-5.

[2] Ishikawa M, Fujii T, Saito M et al. (2006). Int J Gynecol Cancer. 16(1):347-53.

Distributor

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In the event that the user experiences any technical or performance-related issues with the product, please consult the manufacturer or a competent authority.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or the patient is established.

Summary of safety and performance (SSP) can be found in EUDAMED when the related module is available.

Date of publication or revision

2024-11-07
Change(s) made: Complete revision, IVDR certification

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		Gebrauchsanweisung beachten Consult instructions for use
	Hersteller Manufacturer		