Rabbit monoclonal antibody against

PRAME (QR005)

In Vitro Diagnostic Use (IVD)

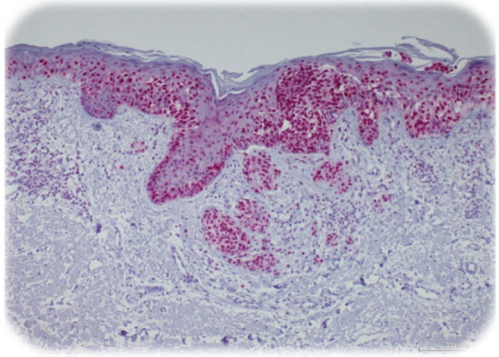

Figure 1 Superficial spreading malignant melanoma stained with anti-PRAME (QR005)

Product identification

C-P003-025	25 µl	Concentrate
C-P003-050	50 µl	Concentrate
C-P003-01	0.1 ml	Concentrate
C-P003-05	0.5 ml	Concentrate
C-P003-10	1 ml	Concentrate
P-P003-30	3 ml	Ready-to-use
P-P003-70	7 ml	Ready-to-use
P-P003-150	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

PRAME is a tumor-associated antigen that is preferentially expressed on the nuclei of neoplastic melanocytes. In normal tissue, expression of this cancer testis antigen is largely restricted to testis.
Anti-PRAME can be used for differential diagnosis of melanoma with diffuse positive nuclear staining versus nevi which are negative or only focally positive for PRAME. Therefore, it can be a helpful additional examination for situations in which antibodies against Hmb-45, Melan A and SOX10 do not provide sufficient information to distinguish between benign and malignant melanocytic lesions[1-5].
Clear cell sarcoma is reported to give consistently negative staining in contrast to malignant melanomas, and can therefore be used for differential diagnosis between clear cell sarcoma (negative) vs. malignant melanoma (positive)[4,11]. Most synovial sarcomas and myxoid liposarcomas are diffusely positive for PRAME[4,11].
Literature using other clones documented that PRAME is expressed not only by melanoma but also by various non-melanoma neoplasms including non-small cell lung cancer (NSCLC), breast carcinoma, renal carcinoma, ovarian carcinoma, and leukemia[10,11].

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-PRAME (QR005)
Host	Rabbit
Subclass	IgG
Immunogen	Synthetic peptide of human PRAME
Antibody concentrate	Concentrated antibody in TRIS (pH 7.4) with < 1 % sera (bovine, donkey) and < 0.1 % sodium azide
Recommended working dilution range	1:50 – 1:100
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:
- Testis (Immature germ cells must show positive nuclear staining)
- Melanoma (Malignant neoplastic melanocytes must show positive nuclear staining)

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:
- Testis (Mature germ cells must show negative nuclear staining)
- Melanoma (Squamous epithelia must show negative nuclear staining)

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 in sebaceous glands.

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***
Testis	2/2

Colon	0/2
Tonsil	0/2
Lymph node	0/1
Liver	0/1

Table 2 Testing of neoplastic FFPE tissue sections

Tissue	***Positive/total cases***
Melanoma	109/111
Nevus	2/51
Myxoid liposarcoma	11/11
Synovial sarcoma	11/11
Clear cell sarcoma of soft tissue	1/10
Melanocytoma	0/51
Uveal melanoma	15/30
Breast carcinoma	3/3
Sebaceous carcinoma	2/2
Periocular sebaceous carcinoma	5/19
Fibrosarcomatous dermatofibrosarcoma protuberans	2/4
NTRK-rearranged spindle cell neoplasm	2/4
Atypical fibroxanthoma	1/7
Kaposi sarcoma	1/5
Intimal sarcoma	7/7
Biphenotypic sinonasal sarcoma	3/3
Angiosarcoma	10/15
Malignant peripheral nerve sheath tumor	9/12
Pleomorphic rhabdomyosarcoma	2/3
Alveolar rhabdomyosarcoma	2/6
Embryonal rhabdomyosarcoma	7/7
Undifferentiated pleomorphic sarcoma	2/12
Leiomyosarcoma	2/15
Low-grade fibromyxoid sarcoma	1/5
Ewing sarcoma	2/10
CIC-rearranged sarcoma	8/8
BCOR-sarcoma	2/5
Thoracic SMARCA4-deficient undifferentiated tumor	5/5

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 QR005 as well as publications dealing with clone QR005 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 111 melanoma[own study,1,4], 11 myxoid liposarcoma[4], 11 synovial sarcoma[4], as well as a total of 51 nevi[own study,1] and 10 clear cell sarcoma of soft tissue[4].
Summarized diagnostic sensitivity, diagnostic specificity, PPV, NPV and LR+ resulted in 98.5%, 95.1%, 97.8%, 96.6% and 20.1 (LR+ > 10 is 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’.
The remaining tested tissues may give an overview of staining features of PRAME (QR005).

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] Gassenmaier M, Hahn M, Metzler G et al. (2021). Cancers (Basel). 13(15):3864.

[2] Koch EAT, Erdmann M, Berking C et al. (2023). Int J Mol Sci. 24(7):6388.

[3] Kunc M, Żemierowska N, Skowronek F, Biernat W (2023). Histopathology. 83(1):3-14.

[4] Cammareri C, Beltzung F, Michal M et al. (2023). Virchows Arch. 483(2):145-56.

[5] Castillo P, Castrejon N, Marginet M et al. (2023). Clin Exp Dermatol. llad405.

[6] Lamas NJ, Lassalle S, Martel A et al. (2023). Pathology. 55(7):929-44.

[7] Mudhar HS, Krishna Y, Cross S et al. (2023). Lab Invest. 104(1):100281.

[8] Salleh AA, Krishna Y & Coupland SE (2023). Ocul Oncol Pathol. 1-8.

[9] Ikeda H, Lethé B, Lehmann F et al. (1997). Immunity. 6(2):199-208.

[10] Lezcano C, Jungbluth AA, Nehal KS et al. (2018). 42(11):1456-65.

[11] Lezcano C, Müller AM, Frosina D et al. (2021). Int J Surg Pathol. 29(8):826-835.

[12] Zhang W, Barger CJ, Eng KH et al. (2016). Oncotarget. 7(29):45352-69.

[13] Iura K, Maekawa A, Kohashi K et al. (2017). Hum Pathol. 61:130-9.

[14] Iura K, Kohashi K, Hotokebuchi Y et al. (2015). J Pathol Clin Res. 1(3):144-59.

[15] Kim SW, Roh J & Park CS (2016). J Pathol Transl Med. 50(6):411-8.

[16] Underwood PA & Bean PA (1985). J Immunol Methods. 80(2):189-97.

[17] Dabbs DJ (2021). Sixth Edition. Elsevier.

[18] Omata M, Liew CT, Ashcavai M & Peters RL (1980). Am J Clin Pathol. 73(5):626-32.

Distributor

quartett Biotechnologie GmbH
Am Mühlenberg 4, 14476 Potsdam, Germany
Tel: +49 (0)30 765 925-0 • Fax: +49 (0)30 765 925-55
service@quartett.com • www.quartett.com

Manufacturer

biocyc Biotechnologie GmbH & Co. KG
Am Mühlenberg 4, 14476 Potsdam, Germany
cert. by TÜV Rheinland LGA Products GmbH
ISO 13485 & ISO 9001
Tel: +49 (0)331 967 826-00

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

2024-06-17
Change(s) made: Complete revision, IVDR certification

Explanation of symbols

REF	Bestellnummer Catalog number		Verwendbar bis Use by
LOT	Chargenbezeichnung Batch code	2 °C 8 °C	Temperaturbegrenzung Temperature limitation
IVD	In Vitro Diagnostika In vitro diagnostic agent		Gebrauchsanweisung beachten Consult instructions for use
	Hersteller Manufacturer		