Polyclonal Rabbit Anti-Human Von Willebrand Factor Code A0082

Intended use

For in vitro diagnostic use.

Polyclonal Rabbit Anti-Human Von Willebrand Factor, Code A0082 is intended for use in immunohistochemistry (IHC). The antibody is a useful tool for studying angiogenesis in the classification of neoplasms such as breast cancer (1) and for the classification of epithelioid hemangioendothelioma (2). Differential classification is aided by the results from a panel of antibodies. 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 qualified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

Synonym for antigen

The previous designation for von Willebrand factor was factor VIII-related antigen (3).

Summary and explanation

von Willebrand factor (vWF) is a large glycoprotein with a multimeric structure and a molecular mass ranging from 500 kDa up to more than 10 000 kDa, the latter being the largest known for a soluble human plasma protein (4). The primary product of the *von Willebrand factor* gene, located at chromosome 12p13.2, is a 2813 amino acid protein comprising a signal peptide of 22 amino acids, a large propeptide of 741 amino acids, and a mature vWF molecule containing 2050 amino acids (5). Expression of the *von Willebrand factor* gene is seen in endothelial cells and megakaryocytes (4, 5). VWF is present in plasma, in the Weibel-Pallade bodies of endothelial cells, in the alpha-granule in megakaryocytes and platelets derived from them, as well as in the subendothelial matrix of the vessel wall. von Willebrand factor serves as a carrier for factor VIII in plasma, protecting the circulating coagulation coenzyme from proteolytic degradation (4).

Refer to *Dako General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: Principle of Procedure; Materials Required, Not Supplied; Storage; Specimen Preparation; Staining Procedure; Quality Control; Troubleshooting; Interpretation of Staining; General Limitations.

Reagent provided

Purified immunoglobulin fraction of rabbit antiserum provided in liquid form. In 0.1 mol/L NaCl, 15 mmol/L NaN_3.

Protein concentration: 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

von Willebrand factor isolated from human plasma.

Specificity

The antibody reacts with human von Willebrand factor/factor VIII complex. Traces of contaminating antibodies have been removed by solid-phase absorption with human plasma proteins. Polyclonal Rabbit Anti-Human Von Willebrand Factor reacts with von Willebrand factor in endothelial cells, megakaryocytes and platelets when tested on formalin-fixed, paraffin-embedded normal human tissues (bone marrow, kidney, liver, lung, lymph node, skin and spleen) (6).

In crossed immunoelectrophoresis using 12.5 µL antibody per cm^2 gel area against 10 µL of human plasma only one precipitate corresponding to the von Willebrand factor appears. Staining: Coomassie Brilliant Blue.

Precautions

1. For in vitro diagnostic use.

2. For professional users.

3. 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.

4. As with any product derived from biological sources, proper handling procedures should be used.

5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.

6. 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 user must verify the conditions. 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 results are observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

Specimen preparation

Paraffin sections: The antibody can be used for labeling paraffin-embedded tissue sections fixed in formalin. Pre-treatment of deparaffinized tissues with proteinase K or heat-induced epitope retrieval is required. For heat-induced epitope retrieval, optimal results are obtained with Dako Target Retrieval Solution, Code S1700, or 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. Less optimal results are obtained with 10 mmol/L citrate buffer, pH 6.0. The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure.

Dako Denmark A/S | Produktionsvej 42 | DK-2600 Glostrup | Denmark | Tel. +45 44 85 95 00 | Fax +45 44 85 95 95 | CVR No. 33 21 13 17

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: Polyclonal Rabbit Anti-Human Von Willebrand Factor, Code A0082, may be used at a dilution range of 1:200-1:400 when applied on formalin-fixed, paraffin-embedded sections of human tonsil and using 20 minutes heat-induced epitope retrieval in Dako Target Retrieval solution, Code S1700, and 30 minutes incubation at room temperature with the primary antibody. The recommended negative control is Dako Rabbit Immunoglobulin Fraction (Solid-Phase Absorbed), Code X0936, diluted to the same protein concentration as the primary antibody. Unless the stability in the actual test system has been established, it is recommended to dilute the product immediately before use or dilute in Dako Antibody Diluent, Code S0809.

Visualization: Dako EnVision+/HRP kits, e.g. Code K4009, are recommended. Follow the procedure enclosed with the selected visualization kit.

Quality control: Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimens.

Staining interpretation

Cells labelled by the antibody display a diffuse or sometimes slightly granular staining in the cytoplasm (6).

Performance characteristics

Normal tissues: The antibody labels endothelial cells lining the lumen of capillaries, lymphatic vessels, arteries and veins, and endothelial cells in glomeruli and in the sinusoids of the liver and spleen. In addition to the labelling of endothelial cells, the antibody labels megakaryocytes and platelets (6).

Abnormal tissues: The antibody has been used to study neovascularization in an invasive breast carcinoma (1). In 99% of 137 cases with epithelioid hemangioendothelioma (EHE) of the liver, the epithelioid, dendritic or mediate cells of the malignant vascular tissue showed strong expression of von Willebrand factor (2). von Willebrand factor is seldom expressed in poorly differentiated vascular tumors (9).

References

1. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma. N Engl J Med 1991;324:1-8.
2. Makhlouf HR, Ishak KG, Goodman ZD. Epithelioid hemangioendothelioma of the liver: a clinicopathologic study of 137 cases. Cancer 1999;85:562-82.
3. Ingerslev J. A sensitive ELISA for von Willebrand factor (vWf:Ag). Scand J Clin Lab Invest 1987;47:143-49.
4. Ruggeri ZM, Ware J. von Willebrand factor. FASEB 1993;7:308-16.
5. Rodeghiero F. von Willebrand disease: still an intriguing disorder in the era of molecular medicine. Haemophilia 2002;8:292-300.
6. Sehested M, Hou-Jensen K. Factor VIII-related antigen as an endothelial cell marker in benign and malignant diseases. Virchows Arch (Pathol Anat) 1981;391:217-25.
7. Yablonka-Reuveni Z. The emergence of the endothelial cell lineage in the chick embryo can be detected by uptake of acetylated low density lipoprotein and the presence of a von Willebrand-like factor. Dev Biol 1989;132:230-40.
8. Smith RA. Evaluation of cross species reactivity of antibodies to human antigens in animal models using immunoperoxidase techniques. J Histotechnol 1990;13:255-69.
9. Leong ASY, Cooper K, Leong FJWM. Manual of diagnostic antibodies for immunohistology. Oxford University Press; 1999. p. 167.

Explanation of symbols

REF	Catalogue number	2 °C 8 °C	Temperature limitation		Manufacturer
IVD	In vitro diagnostic medical device	LOT	Batch code		
	Consult instructions for use		Use by		

Revision 2017.12

Dako Denmark A/S | Produktionsvej 42 | DK-2600 Glostrup | Denmark | Tel. +45 44 85 95 00 | Fax +45 44 85 95 95 | CVR No. 33 21 13 17