

Rat VEGFA Ready-To-Use IHC Kit

Cat.No: IHC0291R
Applications: **IHC-P**
Reactivity: Rat
Size: 50T
Assay type: Immunohistochemistry
Sample type: FFPE tissue
General Information:

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8°C
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8°C, protect from light
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (Rat VEGFA Recombinant Rabbit mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (Goat Anti-Rabbit IgG H&L, HRP conjugated)	6 ml	RTU	2-8°C
7	Chromogen Component A	0.3 ml	RTU	-20°C, protect from light
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (Rat kidney)	1 slide	RTU	RT
12	Datasheet	1 copy		

Storage and Stability: Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

Immunohistochemistry Protocol:

1. Deparaffinization And Rehydration

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. Antigen Retrieval

Add 100× **Antigen Retrieval Buffer** into distilled water to prepare a 1× solution. Boil slides in 1× solution at 95°C-100°C for 15 minutes. Move the slides to 1× solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

3. **Block Endogenous Peroxidase**

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

4. **Serum Blocking**

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

5. **Primary Antibody Incubation**

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **Rat VEGFA Recombinant Rabbit mAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

6. **Secondary Antibody Incubation**

Incubate slides with 2-4 drops of **Goat Anti-Rabbit IgG H&L, HRP conjugated** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

7. **Signal Development**

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer (Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18)** to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

8. **Counterstain**

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. **Dehydration Sheet**

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes:

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.

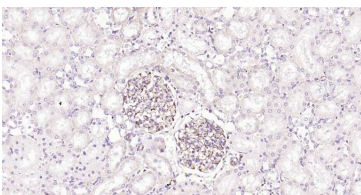
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, please take necessary precautions.
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1× reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as "IHC0291R, Bioss Antibodies". Citation example: "Rat Tissue sections using VEGFA IHC Kit (IHC0291R, Bioss Antibodies) were stained for VEGFA according to the manufacturer's instructions."

Introduction:

VEGF (vascular endothelial growth factor) which is a 45 kDa homodimeric, disulfide-linked glycoprotein involved in angiogenesis which promotes tumor progression and metastasis. VEGF has a variety of effects on vascular endothelium, including the ability to promote endothelial cell viability, mitogenesis, chemotaxis, and vascular permeability. The VEGF family currently includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and PlGF. VEGF and its receptor system have been shown to be the fundamental regulators in the cell signaling of angiogenesis. Most tumors have the absolute requirement of angiogenesis, and VEGF has been described as the most potent angiogenic cytokine linked to this process. To date 5 different isoforms of VEGF have been described. These isoforms are generated as the result of alternative splicing from a single VEGF gene. These various isoforms have been shown to bind to two tyrosine-kinase receptors flt-1 (VEGFR-1) and flk-1/KDR (VEGFR-2), which have been found to be expressed almost exclusively on endothelial cells. VEGF and its high-affinity binding receptors, the tyrosine kinases FLK1 and FLT1, are thought to be important for the development of embryonic vasculature. Studies have shown that an alternately spliced form of FLT1 produces a soluble protein, termed sFLT1, which binds vascular endothelial growth factor with high affinity, playing an inhibitory role in angiogenesis. Elevated levels of VEGF is linked to POEMS syndrome (Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal gammopathy, Skin changes) also known as Crow-Fukase syndrome which affects multiple organs in the body.

Validation Data



Immunohistochemical analysis of paraffin embedded rat kidney tissue slide using IHC0291R (Rat VEGFA IHC Kit).