

# BioSim™ anti-Bevacizumab II (Human) ELISA Kit

(Catalog # E4874-100, 96 assays, Store at 4°C)

## I. Introduction:

Bevacizumab is a humanized IgG1 monoclonal antibody that specifically binds to vascular endothelial growth factor A (VEGF-A) and prevents its binding to cell surface receptors. VEGFA is a pro-angiogenic protein that promotes migration, proliferation, and survival of endothelial cells. Binding of the antibody to VEGFA results in decreased neovascularization, thus limiting blood supply to the tumors and ultimately causes shrinkage of the tumor. The antibody is approved to treat advanced stages of cancer such as colorectal cancer, non-small cell lung cancer, metastatic breast cancer, and glioblastoma multiforme. BioSim™ anti-Bevacizumab ELISA kit has been specifically developed to qualitatively determine antibodies to Bevacizumab in human serum and plasma samples. The ELISA kit is based on the sandwich principle. Controls and samples (serum and plasma) are incubated in the microtiter plate coated with the drug Bevacizumab. After incubation, the wells are washed and the enzymatic activity is detected by the addition of chromogen-substrate. The enzyme-substrate reaction is terminated with an acidic stop solution. The color developed is proportional to the amount of antibodies specific for Bevacizumab present in the samples and controls. The qualitative test results can be evaluated using cut-off value.

## II. Features and Benefits:

- For *in vitro* qualitative determination of antibodies to Bevacizumab in human serum and plasma samples
- Assay Precision: Intra-Assay and Inter-Assay CV < 30%
- Cross Reactivity: Except for Bevacizumab, there is no cross reaction with other therapeutic antibodies and native serum immunoglobins

## III. Sample Type:

Human Plasma and Serum

## IV. Kit Contents:

Components	E4874-100	Part Number
Microtiter Plate	8 X 12 Strips	E4874-100-1
Positive Control	0.3 ml	E4874-100-2
Negative Control	1 ml	E4874-100-3
Assay Buffer	12 ml	E4874-100-4
HRP-conjugate Probe	12 ml	E4874-100-5
TMB substrate (Avoid light)	12 ml	E4874-100-6
Stop Solution	12 ml	E4874-100-7
Wash buffer (20X)	50 ml	E4874-100-8
Plate sealers	2	E4874-100-9

## V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards and sample dilutions
- Absorbent paper

## VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment

## VII. Reagent and Sample Preparation:

**Note:** Before using the kit, spin the tubes and bring down all the components to the bottom of the tubes

1. **Wash Buffer:** Dilute the 20X Wash Buffer to 1X solution using ddH<sub>2</sub>O (10 ml of 20X Wash Buffer + 190 ml of ddH<sub>2</sub>O). Mix the 1X solution thoroughly by manual vortexing. The working stock is stable for 2 weeks after preparation at 4°C. If there are crystals in 20X Wash Buffer, warm the Buffer at 37°C prior to dilution.
2. **Sample preparation:** The usual precautions should be observed for venipuncture. Samples that are hemolytic, icteric or lipemic should be avoided. If the sample is turbid, then it must be centrifuged to separate particulates from solution. Freeze/thawing of serum/plasma samples should be avoided. Drug infusions may interfere with the detection of antibodies to drugs in serum/plasma samples. Hence, it is advisable to take blood samples prior to the scheduled dose. Collected samples can be stored for 2 days at 4°C or 6 months at -20°C

## VIII. Assay Protocol:

**Note:** Bring all the reagents, samples and microtiter plate to room temperature 15 minutes prior to the assay.

It is recommended that all samples be run at least in duplicates.

1. Prepare all reagents and samples as instructed in section VII.
2. Pipette 100 µl of **Assay Buffer** into each of the wells to be used.
3. Add 10 µl of **Negative control** (2 wells), **Positive control** (1 well), and **samples** into appropriate wells. Cover wells and incubate at room temperature (RT) for 60 minutes.
4. Discard the incubation solution. Wash plate 3 times with 300 µl of 1X **Wash Buffer**. Remove excess solution by tapping the inverted

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plate on an absorbent paper.

5. Add 100  $\mu$ l of **HRP-conjugated probe** into each well. Cover the plate with adhesive plate sealer and incubate at RT for 60 minutes.
6. Discard the solution and wash the wells as in step 4.
7. Add 100  $\mu$ l of **TMB substrate** solution and incubate the plate in dark at RT for 20 minutes.
8. Add 100  $\mu$ l of **Stop solution** to stop the reaction. Color changes from blue to yellow.
9. Read the absorbance in micro plate reader set to 450 nm within 30 minutes after pipetting the **Stop solution**. (Use reference wavelength as 650 nm)

**IX. Interpretation of Results:**

For the run to be valid, the OD 450/650 nm of the Positive control should be > 1.500 and the OD 450/650 nm of each Negative control should be < 0.150. If the results do not comply with the aforementioned information, then improper technique or reagent deterioration may be suspected and therefore the assay must be repeated.

The results are evaluated by a cut-off value which is estimated by multiplying the mean OD 450/650 nm of the negative controls by 3

- If "Sample OD450/650 / the mean Negative Control OD450/650" is < 3, the sample is NEGATIVE for Antibody to Bevacizumab.
- If "Sample OD450/650 / the mean Negative Control OD450/650" is  $\geq$  3, the sample is POSITIVE for Antibody to Bevacizumab.

**Note:** The cut-off information provided with this kit can only be considered as a recommendation. Cut-off values must be calculated/set or verified according to scientific standards by the users.

**X. Related Products:**

- BioSim™ Rituximab (Human) ELISA Kit (Cat. No. E4371-100)
- BioSim™ Adalimumab (Human) ELISA Kit (Cat. No. E4372-100)
- BioSim™ Bevacizumab (Human) ELISA Kit (Cat. No. E4373-100)
- BioSim™ Etanercept (Human) ELISA Kit (Cat. No. E4374-100)
- BioSim™ Infliximab (Human) ELISA Kit (Cat. No. E4375-100)
- BioSim™ Trastuzumab (Human) ELISA Kit (Cat. No. E4376-100)
- BioSim™ Golimumab (Human) ELISA Kit (Cat. No. E4377-100)
- BioSim™ Infliximab (Human) ELISA Kit (Cat. No. E4378-100)
- BioSim™ Cetuximab (Human) ELISA Kit (Cat. No. E4379-100)
- BioSim™ Denosumab (Human) ELISA Kit (Cat. No. E4380-100)
- BioSim™ Omalizumab (Human) ELISA Kit (Cat. No. E4381-100)
- BioSim™ Nivolumab (Human) ELISA Kit (Cat. No. E4382-100)
- BioSim™ Pembrolizumab (Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Avelumab (Human) ELISA Kit (Cat. No. E4384-100)
- BioSim™ anti-Rituximab (Human) ELISA Kit (Cat. No. E4385-100)
- BioSim™ anti-Trastuzumab (Human) ELISA Kit (Cat. No. E4386-100)
- BioSim™ anti-Infliximab (Human) ELISA Kit (Cat. No. E4387-100)
- BioSim™ anti-Adalimumab II (Human) ELISA Kit (Cat. No. E4388-100)