Technical Manual

Bevacizumab (Avastin) ELISA Kit

- Catalogue Code: AEFI01151
- Indirect ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Aliases:

Avastin ELISA Kit, Bevacizumab ELISA Kit

Detection method:

Indirect, HRP

Sample Type:

Serum, Plasma, Cell culture supernatant, Cell or tissue lysate, Other liquid samples

Reactivity:

Universal

Range:

0.313-20ng/ml

Sensitivity:

0.188ng/ml

Storage:

2-8°C for 6 months (sealed), please do not freeze!

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this unopened ELISA Kit at 4°C for 6 months. For opened kits, store individual components as described in the Kit Contents section. Date of expiration will be on the ELISA Box label.

3. Description and Principle

Assay Genie ELISA Kits are designed for the precise measurement of analytes in a wide variety of sample types. In response to the increasing demand for high-quality, consistent data from today's scientists, we have developed a range of sensitive, fast, and reliable ELISA kits that meet and exceed these expectations.

The Assay Genie Indirect ELISA kit allows for the quantitative measurement of specific antibodies against Bevacizumab in the following samples: Universal Serum, Plasma, Cell culture supernatant, Cell or tissue lysate, Other liquid samples.

How do our ELISA kits work?

This kit is based on indirect enzyme-linked immune-sorbent assay (ELISA) technology. Antigen is pre-coated onto 96-well plates. The specific antibodies present in the sample and standards bind to the antigen. A secondary antibody conjugated to horseradish peroxidase (HRP) is used as detection antibody. HRP catalyses the TMB substrate to produce a blue colour product that is then changed to yellow after adding the acidic stop solution. The density of yellow colour is proportional to the target amount of sample captured in plate. The concentration of specific antibodies can be calculated by reading the O.D. absorbance at 450nm in a microplate reader and comparing the results with the standard curve. A reference wavelength of 650nm can be used.

4. Kit Contents

The sealed kit can be stored at 2-8 °C. The storage condition for opened kit is specified in the table below. Each kit contains reagents for either 48 or 96 assays including:

No.	Component	48-Well Kit	96-Well Kit	Storage
1	ELISA Microplate (dismountable)	8 x 6	8 x 12	Put the rest of the strips into a sealed foil bag with the desiccant. Store for 1 month at 2-8°C or for 6 months at -20°C
2	Lyophilized Standard	1 vial	2 vials	Put the rest of the standards into a desiccant bag. Store for 1 month at 2-8°C or for 6 months at -20°C
3	HRP-labelled Antibody (Concentrated 100X)	60 uL	120 uL	2-8°C (Avoid Direct Light)
4	TMB Substrate	5 mL	10 mL	2-8°C (Avoid direct light)
5	Sample Dilution Buffer	10 mL	20 mL	2-8°C
6	Antibody Dilution Buffer	5 mL	10 mL	2-8°C
7	Stop Solution	5ml	10ml	2-8°C
8	Wash Buffer (25x)	15 mL	30 mL	2-8°C
9	Plate Sealer	3 pieces	5 pieces	
10	Manual	1	1	

Note: The liquid reagent bottle contains slightly more reagent than indicated on the label.

Please use pipette accurately measure and do proportional dilution.

Additional materials required:

1. 37°C incubator (CO2 incubator for cell culture is not recommenced).
2. Plate Reader with 450nm filter
3. Precision pipettes and disposable pipette tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

Precautions:

1. To determine the concentration of your target, it’s recommended to perform a pilot experiment using standards and a small number of samples.
2. After opening and before use, keep the plate dry.
3. Before using the kit, centrifuge tubes to spin down the standards and antibodies.
4. Store TMB reagents protected from light.
5. Proper washing steps are critical for assay success; any deviations may cause false positives and high background.
6. Use duplicate wells for both standard and sample testing.
7. Do not allow the microplate to dry during the assay, as dry plates can inactivate active components.
8. To avoid cross-contamination, do not reuse tips and tubes.
9. Avoid mixing reagents from different batches

5. Sample Preparation

General considerations: According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C). For optimal results, avoid repeated freeze-thaw cycles.

Serum: If using serum separator tubes, allow samples to clot for 30 minutes at room temperature. Centrifuge for 20 minutes at 1,000x g. Collect the serum fraction and assay promptly or aliquot and store the samples at -20 or -80°C. Avoid multiple freeze-thaw cycles.

If serum separator tubes are not being used, allow samples to clot overnight at 2-8°C. Centrifuge for 20 minutes at 1,000x g. Remove serum and assay promptly or aliquot and store the samples at -20 or -80°C. Avoid multiple freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 4°C for 15 mins at 1000 × g within 30 mins of collection. Collect the plasma fraction and assay promptly or aliquot and store the samples at -20 or -80°C. Avoid multiple freeze-thaw cycles.

Note: Over haemolyzed samples are not suitable for use with this kit.

Cell culture supernatant: Collect the supernatant: Centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect clarified cell culture supernatant to detect immediately. assay promptly or aliquot and store the samples at -80°C assay.

Cell lysates:

Suspension Cell Lysate:

1. Centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect the cell.
2. Add pre-cooling PBS into collected cell and gently mix; Centrifuge at 2500 rpm at 2 -8℃ for 5 minutes, then collect the cell.
3. Add 0.5-1ml of RIPA lysis buffer (medium). The RIPA lysis buffer should have a pH of 7.3. It is not recommended to use components that contain NP-40 lysis buffer, Triton X-100 surfactant, or DTT with higher concentrations, as they may interfere with the antigen-antibody reaction. Alternatively, you can use a solution of 50mM Tris, 0.9% NaCl, and 0.1% SDS at pH 7.3. Add some protease inhibitors, such as PMSF, at a working concentration of 1mmol/L. To lyse the cells, keep them on ice for 30 minutes to 1 hour. During the lysate process, either use a pipette tip for pipetting or intermittently shake the centrifugal tube to ensure complete protein lysis. If sticky DNA appears, ultrasound can be used to disrupt it. (Another option for processing the sample on ice is to use an ultrasonic condition with a 3-

5mm probe, 150- 300W power, 3-5 seconds per cycle, and 30 seconds intervals for 1-2 seconds of working time.)

4. After lysate or ultrasonic disruption, centrifuge the mixture at 10000rpm at 2-8℃ for 10 minutes. Then, transfer the supernatant into an EP tube. The assay should be performed immediately, or the sample can be stored at -20 or -80℃.

Adherent Cell Lysate:

1. Remove the supernatant and wash three times with pre-cooled PBS.
2. Add 0.5-1ml RIPA lysis buffer and some protease inhibitors (read requirements in suspension cell). Scrape adherent cell gently with a cell scraper.
3. Add the cell suspension to a centrifuge tube. Lyse the cells on ice for 30 minutes to 1 hour. Alternatively, disrupt the cells using ultrasound (refer to the requirements for suspension cells).
4. After lysate/ultrasonic disruption, centrifuge the mixture at 10,000rpm for 10 minutes. Then, transfer the supernatant into an EP tube. The assay should be performed immediately, or the sample can be stored at -20 or -80℃.

Notes: It is recommended to use ultrasound to disrupt cells during cell lysate preparation. Ultrasound can efficiently break DNA. DNA fragments will not significantly interfere with the performance of the ELISA kit.

Tissue Homogenates: Generally, tissue samples are required to be made into homogenization.

1. Place the target tissue on ice. Remove residual blood by washing tissue with pre-cooled PBS buffer (0.01M, pH=7.4). Then weigh for usage.

2. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue. Usually, 9mL PBS would be appropriate to 1 gram tissue pieces. It is recommended to add some protease inhibitors into the PBS (e.g. 1mM PMSF).

3. The samples can be further processed using ultrasonic disruption or freeze-thaw cycles (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.) to get the homogenates.

4. Centrifuge the homogenates for 5 minutes at 5000×g. Collect the supernatant to detect immediately or aliquot the supernatant and store it at -20°C or -80°C for future's assay.

5. Determine total protein concentration by BCA assay kit (MAES0177) for further data analysis. Usually, total protein concentration for Elisa assay should be within 1-3mg/ml. Some tissue samples such as liver, kidney, pancreas which containing a higher endogenous

peroxidase concentration may react with TMB substrate causing false positivity. In that case, try to use 1% H_2O_2 for 15min inactivation and perform the assay again.

Notes: PBS buffer or the mild RIPA lysis can be used as lysates. While using RIPA lysis, make the PH=7.3. Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT due to their severe inhibition for kits' working. We recommend using 50mM Tris+0.9%NaCL+0.1%SDS, PH7.3.

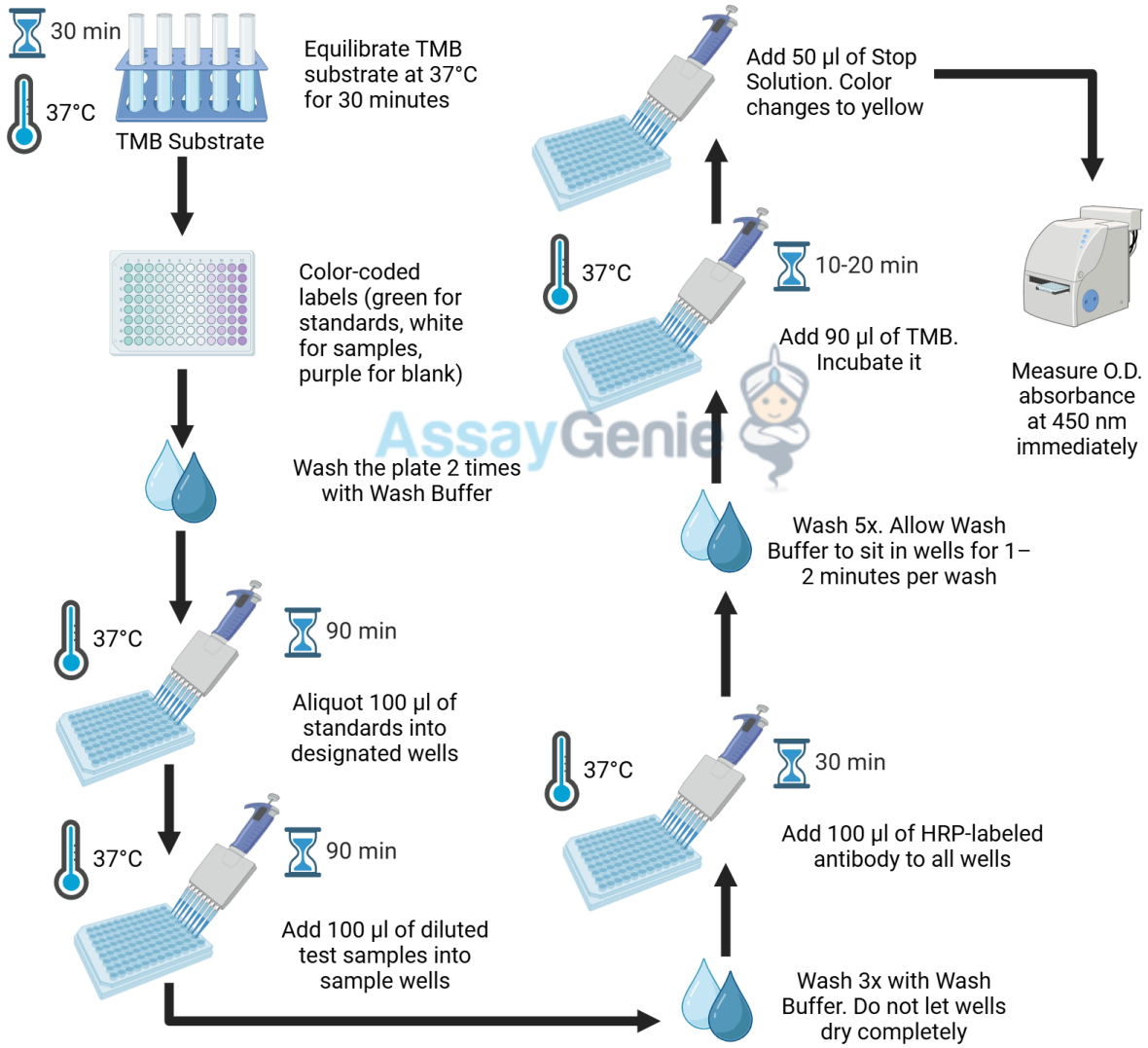
Other Biological Samples: Centrifuge samples for 15 minutes at 1000 x g (4°C). Collect the supernatant to detect immediately. Or aliquot the supernatant and store it at -80°C.

Notes for Samples

1. Blood Collection Tubes: Use disposable, non-endotoxin blood collection tubes. Avoid using hemolyzed or lipemic samples.
2. Optimal Sample Storage Conditions: Store samples at 2-8°C for up to 5 days. Store at -20°C for up to 6 months. Store at -80°C for up to 2 years. For long-term storage, samples can be stored in liquid nitrogen. When thawing frozen samples, use a rapid water bath (15-25°C) to minimize the formation of ice crystals at 0°C. After thawing, centrifuge the samples to remove any precipitate, then mix well.
3. Detection Range: The detection range of this kit does not directly correspond to the analyte concentration in the sample. For samples with higher or lower concentrations, dilution or concentration may be necessary.
4. Pretest Recommendation: For special samples with no reference data, a pretest is recommended to validate the assay's accuracy and reliability.
5. Recombinant Protein Compatibility**:** Recombinant proteins may not bind effectively to the capture or detection antibodies in the kit, which could result in undetectable assay results.

6. Workflow Overview

FI Indirect ELISA Kit (HRP Quantitative) workflow



7. Standard and Reagent Preparation

Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350µl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate with 350µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute (Note: set the height of the needles; be sure the fluid can be taken up completely).

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

Note: Matrix components in the serum/plasma samples will affect test results, samples need to be diluted at least ½ with Sample Dilution Buffer before testing.

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use.

1. Wash Buffer:

Dilute 30ml (15 ml for 48 well assay) of Concentrated Wash Buffer into 750 ml (375 ml for 48 well assay) of Wash Buffer with deionized or distilled water. Store unused solution at 4°C for 48 hs. If crystals have formed in the concentrate, warm at 40°C in water bath (Heating temperature should not exceed 50°C) and mix gently until crystals have completely dissolved. The solution should be cooled to room temperature before use.

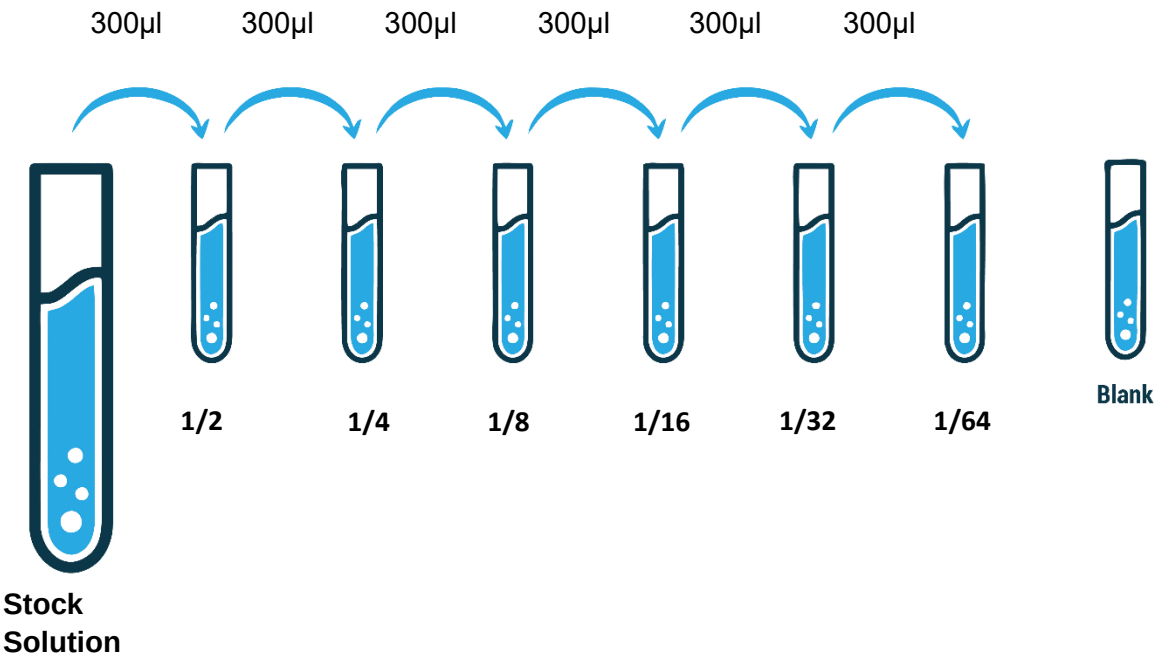
2. Standard Dilution:

1). Centrifuge standard tube for 1min at 10000xg. Add 1ml of Sample dilution buffer into one Standard tube (labelled as Stock Solution),), keep the tube at room temperature for 2 min Invert the tube several times to mix gently. (Or you can mix it using a low speed vortex mixer for 3-5 seconds.)

Note: If the standard vial concentration is different to the highest value in the range (please see page 3), please dilute using sample buffer to match highest range value to create stock solution.

2). Label 7 Eppendorf tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Aliquot 300µl of the Sample dilution buffer into each tube. Add 300µl of the above (Stock Solution) standard solution into 1st tube and mix thoroughly. Transfer 300µl from 1st tube to 2nd tube and mix thoroughly. Transfer 300µl from 2nd tube to 3rd tube and mix thoroughly, and so on.

DILUTION SERIES



Note: The standard solutions are best used within 2 hours. The standard solution series should be kept at 4°C for up to 12 hours.

3. Preparation of HRP-labelled antibody working solution:

Prepare within 30 in before the experiment. This solution should not be stored for extended periods.

1. Calculate the total volume of the working solution: 100µl / well × quantity of wells (it's better to prepare 100-200µl more than the total volume).
2. Centrifuge for 1 min at 1000xg.
2. Dilute the HRP-detection antibody with antibody dilution buffer at 1:100 and mix thoroughly (i.e. Add 10µl of antibody into 990µl of antibody dilution buffer.)

8. Assay Procedure

Before adding to the wells, equilibrate the TMB substrate for at least 30 mins at 37'C When diluting samples and reagents, ensure they are mixed completely and evenly. It is recommended to plot a standard curve for each test.

1. Set standard, test sample (diluted at least ½ with Sample Dilution Buffer) and control (blank) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard, sample and control (blank) wells!**
2. **Standards:** Aliquot 100µl of standard solutions into the standard wells.
3. **Samples:** Add 100µl of properly diluted samples into the sample wells.
4. **Incubation:** Seal the plate with a cover and incubate at 37 °C for 90 mins.
5. **Wash:** Remove the cover, and wash plate 3 times with Wash Buffer according to instructions. **Do NOT let the wells dry completely at any time.**
6. **HRP-labelled antibody:** Add 100µl of HRP-labelled antibody working solution into the bottom of each well (standard, test sample & zero wells) without touching the side walls.
7. **Incubation:** Seal the plate with a cover and incubate at 37°C for 30 mins.
8. **Wash:** Remove the cover, and wash plate 5 times with Wash buffer. **Let the Wash Buffer stay in the wells for 1-2 minutes for each wash.**
9. **TMB Substrate:** Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37°C in dark for 10-20 mins. (Note: This incubation time is for reference only, the optimal time should be determined by the end-user.) As soon as a blue colour

develops in the first 3-4 wells (with most concentrated standards) and the other wells show no obvious colour, terminate the reaction by moving to Step 10.

10. **Stop Solution:** Add 50µl of Stop solution into each well and mix thoroughly. The colour changes into yellow immediately.

11. Read the O.D. absorbance at 450 nm in a pre-heated microplate reader immediately after adding the stop solution.

9. Data Analysis

Calculate the mean O.D.450 value of the duplicate reading of each standard, control and sample.

Then calculate the relative O.D.450 using the following equation:

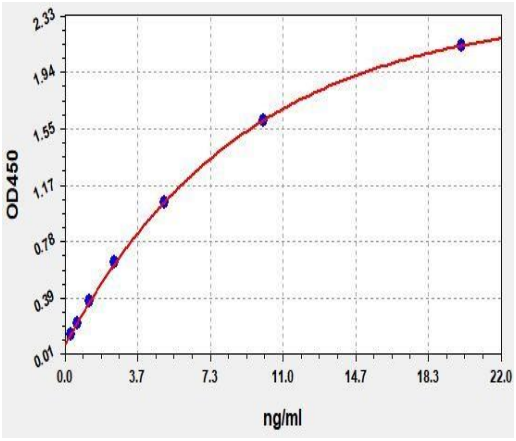
The relative O.D.450 = (the O.D.450 of each well) – (the O.D.450 of Zero well)

The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). (Do not plot the blank). The concentration of the samples can be determined from the standard curve. It is recommended to use professional software such as curve expert 1.3 or 1.4. to create a four parameter logistic curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

10. Typical Data & Standard Curve

Results of a typical standard run of this ELISA are shown below. This standard curve was generated at our lab for demonstration purpose only. **Each user should obtain their own standard curve as per experiment.**



Specificity

This assay has high sensitivity and excellent specificity for detection of Bevacizumab . No significant cross-reactivity or interference between Bevacizumab and analogues was observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between Bevacizumab and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with a certain level of Bevacizumab and the recovery rates were calculated by comparing the measured value to the expected amount of Bevacizumab in the samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	87-102	93
EDTA Plasma(n=5)	89-100	97
Heparin Plasma(n=5)	88-101	95

Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of Bevacizumab and their serial dilutions.

Sample	1:2	1:4	1:8
Serum(n=5)	88-105%	87-103%	87-104%
EDTA Plasma(n=5)	85-99%	85-99%	82-100%
Heparin Plasma(n=5)	83-100%	81-95%	81-100%

Precision

Intra-assay Precision: samples with low, medium and high concentration were tested 20 times on same plate.

Inter-assay Precision: samples with low, medium and high concentration were tested 20 times on three different plates.

Stability

The stability of the Bevacizumab ELISA Kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage conditions.

Standard (n=5)	37°C for 1 month	4°C for 6 months
Average (%)	80	95-100

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end**.**

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

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