Technical Manual

LPS (Lipopolysaccharides) ELISA Kit (UNFI0091)

- Catalogue Code: UNFI0091
- Competitive ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Aliases:

LPS, Lipopolysaccharides

Detection method:

Competitive, Double Antigen

Sample Type:

Serum, Plasma and other biological fluids

Reactivity:

Universal

Range:

0.313-20ug/ml

Sensitivity:

< 0.188ug/ml

Storage:

2-8°C for 6 months. After the biotin-labeled antigen is dissolved, please divide it into several small packages and freeze them at -20 °C. If the biotin-labeled antigen is removed from -20°C should be stored at 2-8°C and used up within 2 weeks.

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit at 4°C. Date of expiration will be on the ELISA Box label.

3. Description and Principle

The Assay Genie Sandwich ELISA kit is a highly sensitive assay for the quantitative measurement of a specific analyte in the following samples: serum, blood, plasma, cell culture supernatant and other related supernatants and tissues.

How do our ELISA kits work?

The Assay Genie (enzyme-linked immunosorbent assays) assay kits are designed for the quantitative measurement of analytes in a wide variety of samples. As today's scientists demand premium quality, consistent data, Assay Genie have developed a range of sensitive, fast and reliable ELISA kit assays to meet and exceed those demands. Our assay kits use a quantitative sandwich ELISA technique and each kit comes with highly specific antibodies pre-coated onto a 96-well microtiter plate.

At Assay Genie we understand the need for speed! Therefore, we have developed an ultrafast protocol for rapid results. Once you have prepared and plated your samples, blanks and standards, you simply incubate with the specific biotin-conjugated primary Antigen and Avidin conjugated Horseradish Peroxidase (HRP). After plate washing and addition of the TMB (3,3',5,5'-Tetramethylbenzidine) solution, the appearance of a blue colour is detected due to an enzymatic reaction catalysed by HRP. Next the addition of the Stop Solution terminates the HRP reaction and the blue colour turns yellow with the signal intensity measured on a plate reader at 450nm. The amount of bound analyte is proportional to the signal generated by the reaction meaning the kit assay gives you a quantitative measurement of the analyte in your samples.

4. Kit Contents

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	2-8°C/-20°C
2	Lyophilized Standard	1 vial	2 vials	2-8°C/-20°C
3	Sample Dilution Buffer	10 mL	20 mL	2-8°C
4	Biotin-labeled Antigen (Lyophilized)	1 vial	1 vial	2-8°C (Avoid Direct Light)
5	Antigen Dilution Buffer	5 mL	10 mL	2-8°C
6	HRP-Streptavidin Conjugate (SABC)	60 uL	120 uL	2-8°C (Avoid Direct Light)
7	10 mM PBS	200 uL	200 uL	2-8°C
8	SABC Dilution Buffer	5 mL	10 mL	2-8°C
9	TMB Substrate	5 mL	10 mL	2-8°C (Avoid direct light)
10	Stop Solution	5ml	10ml	2-8°C
11	Wash Buffer (25x)	15 mL	30 mL	2-8°C
12	Plate Sealer	3 pieces	5 pieces	
13	Manual	1	1	

Additional materials required:

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

Precautions:

1. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.

2. Ensure unopened and unused plate is kept dry to avoid contamination.

3. Before using the kit, centrifuge tubes to spin down standard & antibodies.

4. Avoid light for storage of TMB reagents.

5. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

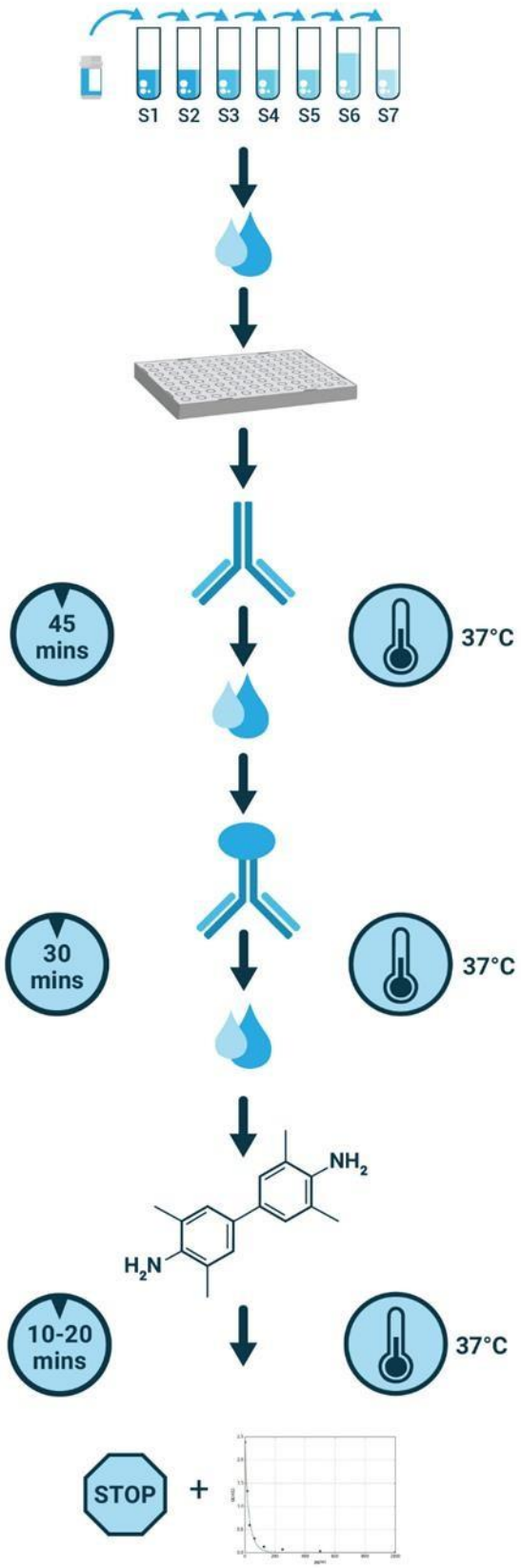
6. Duplicate wells are recommended for both standard and sample testing.

7. Do not let the microplate wells dry during assay. Dry plates will inactivate active components.

8. Do not reuse tips and tubes to avoid cross contamination.

9. Avoid using the reagents from different batches together.

5. Workflow Overview



Prepare plate standards and reagents

Wash plate x2

Add 100µl of samples, standards and controls

Add 100µl Biotin-labeled antigen working solution

Wash plate x3

Add 100µl SABC working solution

Wash plate x 5

Add 90µl TMB Substrate

Add 50µl STOP solution and read absorbance @ 450nm

6. 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 -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 -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 -80°C. Avoid multiple freeze-thaw cycles.

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

Adherent and Suspension Cell Culture: Use three T25 flasks or one T27 flask for cell culture. Cell number is ($1x10^7$).

1. Suspension Cells: Centrifuge at 2500 rpm for 5 minutes (2-8°C). Collect clarified cell culture supernatant.

2. Adherent Cells: Collect supernatant directly. Centrifuge at 2500 rpm for 5 minutes (2-8°C). Collect clarified cell culture supernatant for immediate detection or store it separately (-80°C).

Cell Lysate Preparation: There are two types of cell lysate specified below.

1. Suspension Cell Lysate: Centrifuge at 2500 rpm for 5 minutes (2-8°C). Add pre-cooling PBS and gently mix. Recollect cells by centrifugation. Add 0.5-1 ml RIPA lysis buffer (NP-40 lysis buffer or Triton X-100 surfactant are not recommended due to their interference with the antigen-Antigen reaction). Add a suitable protease inhibitor (e.g. PMSF, working concentration: 1 mmol/L). Lyse cells on ice for 30 min-1 h. During the lysate process, use the pipette tip or intermittent shaking of the centrifuge tube to completely lyse the protein. Alternatively, subject cells to fragmentation by ultrasonic generator (14 μM for 30 s) or ultrasonic cell disruptor (300 W, 3~5 s/time. 30 s intervals, four-five times). At the end of the

lysate or ultrasonic disruption, centrifuge at 10000 rpm for 10 minutes (2-8°C). Add the supernatant into an EP tube and store (-80°C).

2. Adherent Cell Lysate: Absorb the supernatant and add pre-cooling PBS. Add 0.5-1 ml RIPA lysis buffer (NP-40 lysis buffer or Triton X-100 surfactant are not recommended due to their interference with the antigen-Antigen reaction). Add a suitable protease inhibitor (e.g. PMSF, working concentration: 1 mmol/L). Scrape the adherent cells gently with a cell scraper. Add the cell suspension into the centrifuge tube. Lyse cells on ice for 30 min-1 h. During the lysate process, use the pipette tip or intermittent shaking of the centrifuge tube to completely lyse the protein. Alternatively, subject cells to fragmentation by ultrasonic generator (14 μM for 30 s) or ultrasonic cell disruptor (300 W, 3~5 s/time. 30 s intervals, four-five times). At the end of the lysate or ultrasonic disruption, centrifuge at 10000 rpm for 10 minutes (2-8°C). Add the supernatant into an EP tube and store (-80°C).

Other Biological Fluids: Centrifuge samples at 1000xg for 20 minutes (2-8°C). Collect the supernatant and immediately carry out the assay.

Tissue Homogenates: Rinse tissue with 1X PBS to remove excess blood. Mince tissue after weighing it and homogenize in PBS (the volume depends on the weight of the tissue. 9mL PBS (including protease inhibitors) would be appropriate for 1 gram of tissue. To further lyse cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000 x g and the supernatant is removed for assaying. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.

Notes

Samples stored at 2-8°C should be used within 5 days, samples stored at -20°C should be assayed within 1 month and samples stored at -80°C should be assayed within 2 months to reduce the loss of bioactivity. Avoid multiple freeze-thaw cycles. Hemolysed samples are not suitable for this assay.

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 sample 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 30 ml (15 ml for 48T) of Concentrated Wash Buffer into 750 ml (375 ml for 48T) of Wash Buffer with deionized or distilled water. Store unused solution at 4°C. 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. Preparation of Biotin- labeled Antigen

a) Dissolve: Add 100ul 10mM PBS into the tube and mix them thoroughly, divide it into several small vials and store the remaining reagent at -20°C.
b) Calculate required total volume of the working solution: 55μl /well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)
c) According to the volume required for this experiment, dilute the Biotin- labeled Antigen with Antigen Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1μl of Biotin- labeled Antigen into 99μl of Antigen Dilution Buffer.)

After the biotin-labeled antigen is dissolved, please divide it into several small packages and freeze them at -20 °C. The biotin-labeled antigen removed from -20 °C should be stored at 2-8°C and used up within 2 weeks.

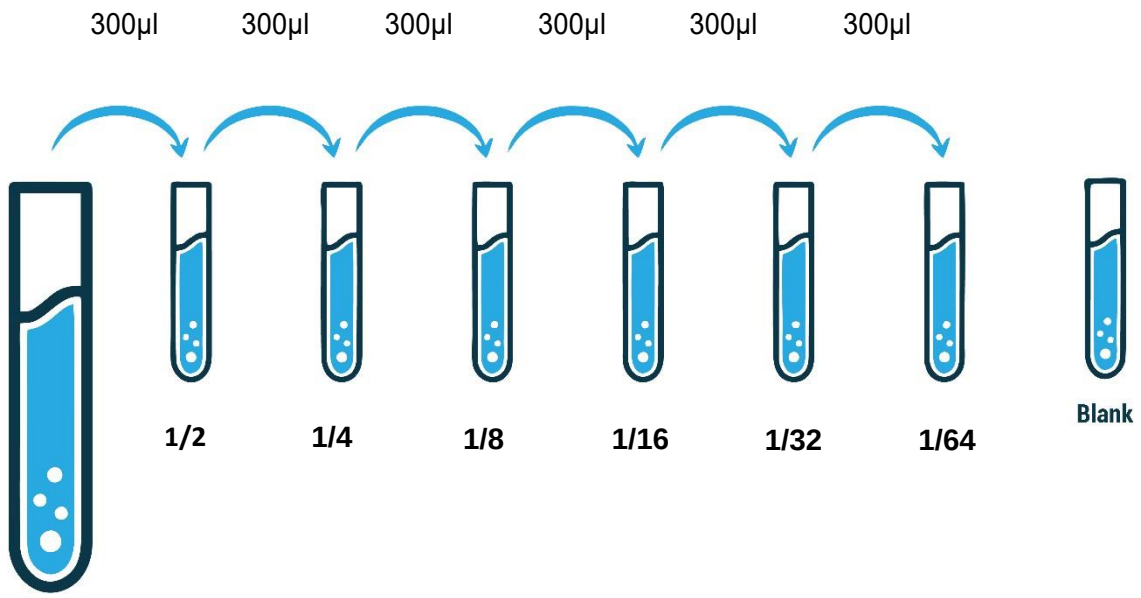
2. Standards:

1). Add 0.5 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly.

Note: If the standard tube concentration higher than the range of the kit, please dilute it and labeled as zero tube.

2). Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the Sample Dilution Buffer into each tube. Add 0.3ml of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control.

DILUTION SERIES



Stock Solution

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. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Preparation of Standard/Sample/Biotin-Antigen compound:

Prepare within 30 minutes before the experiment.

1) When samples and standards are not tested in duplicate:
   a) Take 55ul prepared standard of each gradient and 55ul biotin-labeled antigen working Solution and mix them well in EP tube for later use.
   b) Take 55ul diluted sample and 55ul biotin-labeled antigen working Solution and mix well in EP tube for later use.

2) When the sample and standard is tested in duplicate:
   a) Take 110ul prepared standard of each gradient and 110ul biotin-labeled antigen working Solution and mix them well in EP tube for later use.
   b) Take 110ul diluted sample and 110ul biotin-labeled antigen working Solution and mix well in EP tube for later use.

4. Preparation of HRP-Streptavidin Conjugate (SABC) working solution:

Prepare within 30 minutes of starting the experiment.

1. Calculate the total volume of the working solution: 100μl / well × quantity of wells. (Allow 100-200μl more than the total volume)

2. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1μl of SABC into 99μl of SABC 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 (zero) 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. **Biotin-labeled Antigen:** Add 100ul Standard/Sample/Biotin-Antigen compound into each well. (Solutions are added to the bottom of microplate well, avoiding inside wall touching and foaming as much as you can.)

3. **Incubate:** Seal the plate with a cover and incubate at 37°C for 45 mins.

4. **Wash:** Remove the cover, and wash plate 3 times with Wash buffer. **Let the Wash Buffer stay in the wells for 1-2 minutes for each wash.**

5. **HRP-Streptavidin Conjugate (SABC):** Add 100µl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 mins.

6. **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.**

7. **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.

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

9. **OD Measurement:** Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

9. Data Analysis

Calculate using the following equation:

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

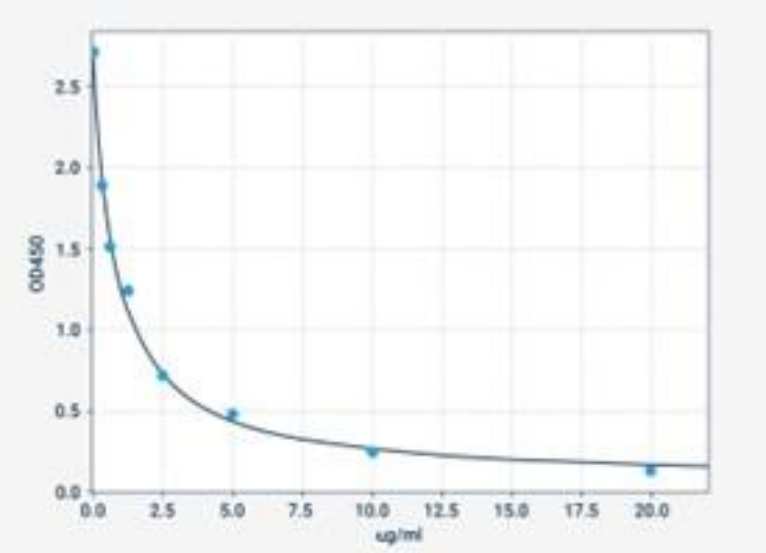
The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be determined from the standard curve. It is recommended to use professional software such as curve expert 1.3 or 1.4.

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

Standard Curve

Results of a typical standard run of a ELISA kit 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 LPS. No significant cross-reactivity or interference between LPS and analogues was observed.

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

Recovery

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

Matrix	Recovery Range (%)	Average (%)
bacteria culture medium(n=5)	85-101	97

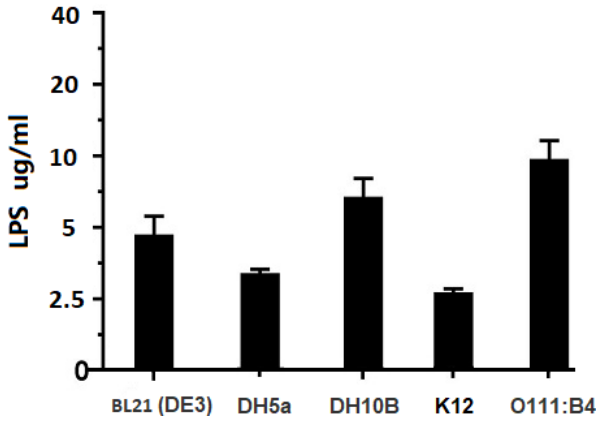
Linearity

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

Sample	1:2	1:4	1:8
bacteria culture medium (n=5)	85-103%	88-94%	86-104%

Sample Test Data

1 gram of E. coli was mixed with 1ml PBS and then broken by ultrasound, the samples were centrifuged for 20 minutes at 10000rpm at 2-8°C. Collect supernatant and carry out the assay immediately.



Precision

- **Intra-Assay:** CV<8%
- **Inter-Assay:** CV<10%

Stability

The stability of the LPS 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**.**

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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

Email: info@ASSAYGenie.com

Web: www.ASSAYGenie.com