



## Technical Manual

### Human OCT4 (Octamer Binding Transcription Factor 4) ELISA Kit

- **Catalogue Code: HUF101494**
- **Sandwich ELISA Kit**
- **Research Use Only**

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## 1. Key features and Sample Types

### Aliases:

OCT4, Octamer Binding Transcription Factor 4, OCT4, POU domain, class 5, transcription factor 1, Octamer-binding transcription factor 3, OTF-3, Octamer-binding protein 3, Oct-3, Octamer-binding protein 4, Oct-4, OCT3, OTF3, OTF4

### Uniprot:

Q01860

### Detection method:

Sandwich, Double Antibody

### Sample Type:

Serum, Plasma and other biological fluids

### Reactivity:

Human

### Range:

0.156-10ng/ml

### Sensitivity:

< 0.094ng/ml

### Storage:

2-8°C for 6 months

### Expiry:

See Kit Label

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## 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.

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### 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 antibody 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 Antibody (Concentrated, 100X)	60 uL	120 uL	2-8°C (Avoid Direct Light)
5	Antibody Dilution Buffer	5 mL	10 mL	2-8°C
6	HRP-Streptavidin Conjugate (SABC, 100X)	60 uL	120 uL	2-8°C (Avoid Direct Light)
7	SABC Dilution Buffer	5 mL	10 mL	2-8°C
8	TMB Substrate	5 mL	10 mL	2-8°C (Avoid direct light)
9	Stop Solution	5ml	10ml	2-8°C
10	Wash Buffer (25x)	15 mL	30 mL	2-8°C
11	Plate Sealer	3 pieces	5 pieces	
12	Manual	1	1	

### Additional materials required:

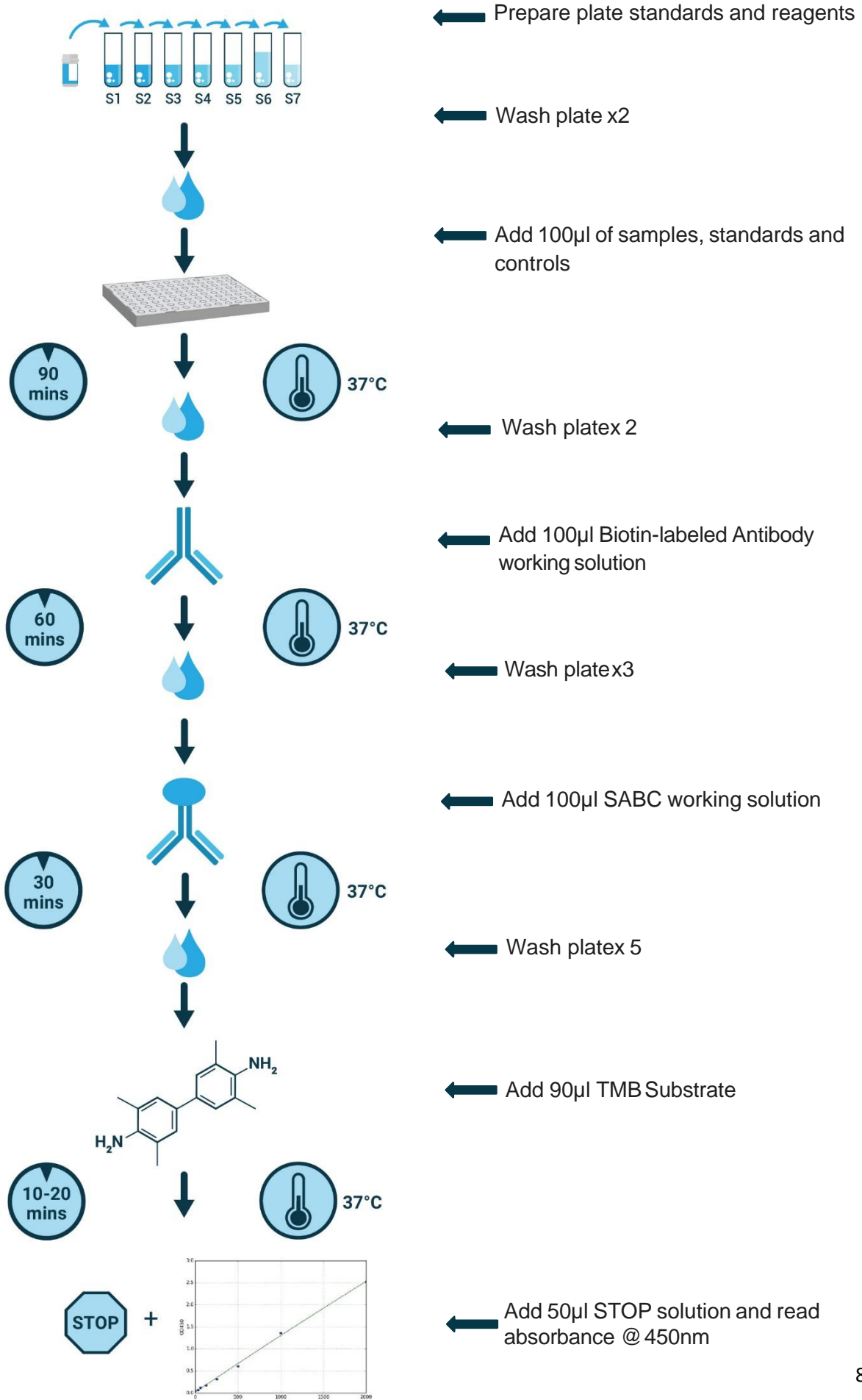
1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer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

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### 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 plates are kept dry to avoid contamination.
3. Before using the kit, centrifuge tubes 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



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## 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.

**Cell culture supernatant:** Collect the cell culture media by pipette, followed by centrifugation at 4°C for 5 mins at 2500 rpm. Collect the clear supernatant and assay immediately.

### Tissue Homogenates:

Generally, tissue samples are homogenized. Protocol is as below:

1. Place the target tissue on ice. Remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Then weigh for use.
2. Use lysate to grind tissue homogenates on ice. The volume of lysate added depends on the weight of the tissue. Usually, 9mL PBS would be appropriate for 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS (e.g. 1mM PMSF).
3. Use ultrasonic disruption or freeze-thaw cycles to further process the sample (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.)
4. Homogenates are then centrifuged for 5 minutes at 5000xg. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future assays.
5. Determine total protein concentration using a BCA kit. The total protein concentration should be within 1-3mg/ml. Some tissue samples such as liver, kidney, and pancreas contain a higher endogenous peroxidase concentration may react with TMB substrate causing false positivity. In that case, use 1% H<sub>2</sub>O<sub>2</sub> for 15min inactivation and perform the assay again.



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**Notes:** PBS or mild RIPA lysis buffer can be used as lysates. When using RIPA lysis buffer, the pH = 7.3. Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT. We recommend using 50mM Tris+0.9%NaCL+0.1%SDS, pH7.3.

### Cell lysates:

1. **Suspension Cell Lysate:** Centrifuge at 2500 rpm at 2-8°C for 5 minutes and collect cells. Then add precooling PBS into collected cells and mix gently. Recollect cells by repeating centrifugation. Add 0.5-1ml cell lysate and an appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Lyse the cells on ice for 30min-1h or disrupt the cells by ultrasonic disruption.

2. **Adherent Cell Lysate:** Absorb supernatant and add pre-cooling PBS and wash three times. Add 0.5-1ml cell lysate and an appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Scrape the adherent cells with a cell scraper. Lyse the cell suspension in the centrifuge tube on ice for 30min-1h or disrupt the cells by ultrasonic disruption.

3. During the lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Any viscous product is DNA which can be disrupted by ultrasonic cell disruptor on ice. (3~5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working).

4. At the end of lysate or ultrasonic disruption, centrifuge at 10000rpm at 2-8°C for 10 minutes. Then, the supernatant is added into EP tube to detect immediately. Or you can aliquot the supernatant and store it at - 80°C for future assays.

### Other Biological Samples:

Centrifuge samples for 15 minutes at 1000×g at 2-8°C. Collect the supernatant to detect immediately. Alternatively, you can aliquot the supernatant and store it at -80°C for future assays.

### 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.

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## Recommended Sample Dilution Ratio

If you are running the assay in duplicate, please follow the calculation: volume of sample and diluent x number of duplicate well)

**For 2-fold dilution (1/2):** One step dilution. Add 60ul sample into 60ul sample diluent and mix gently.

**For 5-fold dilution (1/5):** One step dilution. Add 24ul sample into 96ul sample diluent and mix gently.

**For 10-fold dilution (1/10):** One step dilution. Add 12ul sample into 108ul sample diluent and mix gently.

**For 20-fold dilution (1/20):** One step dilution. Add 6ul sample into 114ul sample diluent and mix gently.

**For 50-fold dilution (1/50):** One step dilution. Add 3ul sample and 47ul normal saline (0.9% NaCl) into 100ul sample diluent and mix gently.

**For 100-fold dilution (1/100):** One step dilution. Add 3ul sample and 177ul normal saline into 120ul sample diluent and mix gently.

**For 1000-fold dilution (1/1000):** Two step dilution. Create a 50-fold dilution first (normal saline is used throughout the dilution). Then, create a 20-fold dilution and mix gently.

**For 10000-fold dilution (1/10000):** Two step dilution. Create a 100-fold dilution first (normal saline is used throughout the dilution). Then, create the same dilution again and mix gently.

**For 100000-fold dilution (1/100000):** Three step dilution. Create a 50-fold dilution and 20-fold dilution respectively (normal saline is used in the first two steps.) Finally, create a 100-fold dilution and mix gently.

**Notes:** The volume in each dilution is not less than 3ul. Dilution factor should be within 100-fold. Mixing during dilution is required to avoid foaming.

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## 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.

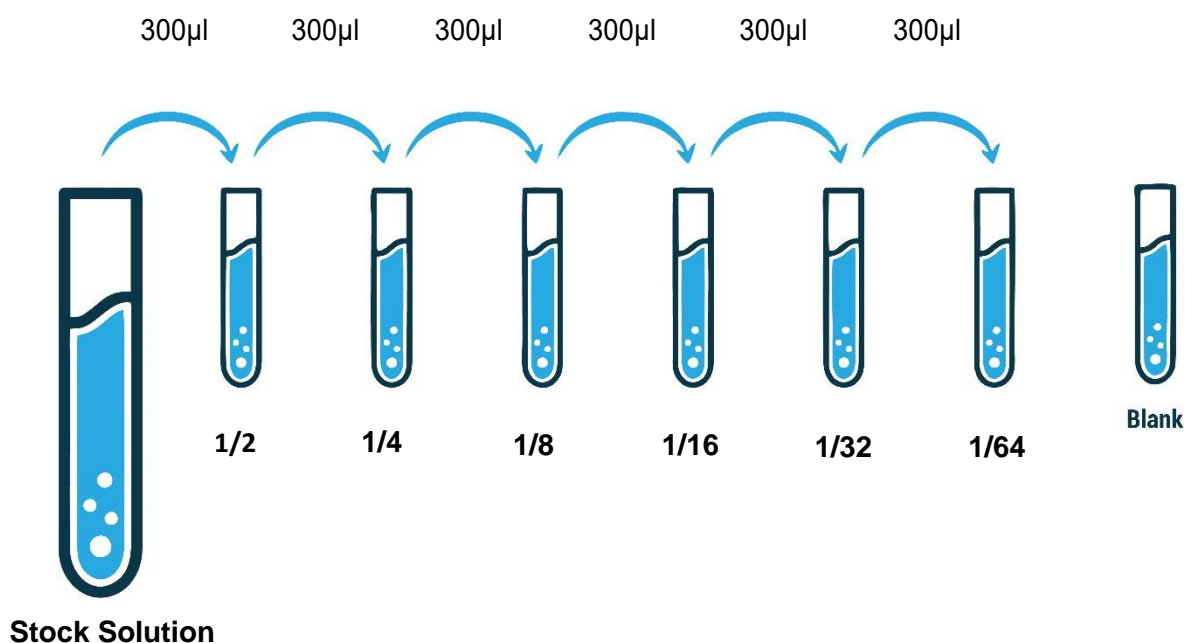
## 2. Standard Dilution:

1). Add 1ml of Sample dilution buffer into one Standard tube (labelled as Stock Solution), keep the tube at room temperature for 10 min and mix thoroughly.

**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. Sample dilution buffer is used as blank control.

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

## 3. Preparation of biotin-labeled Antibody working solution:

Prepare within 1 hour before the experiment.

1. Calculate the total volume of the working solution: 100µl / well x quantity of wells (Allow 100-200µl more than the total volume).
2. Centrifuge for 1min at 1000xg low speed bringing the concentrated biotin-labeled antibody to the bottom of the tube.

3. Dilute the biotinylated detection antibody with antibody dilution buffer at 1:99 and mix them thoroughly. (e.g., Add 10ul concentrated biotin-labeled antibody into 990ul antibody dilution buffer.)

#### 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:  $100\mu\text{l} / \text{well} \times \text{quantity of wells}$ . (Allow 100-200 $\mu\text{l}$  more than the total volume)
2. Centrifuge for 1min at 1000xg low speed bringing the concentrated SABC to the bottom of the tube.
3. Dilute the concentrated SABC with SABC dilution buffer at 1:99 and mix them thoroughly. (e.g. Add 10ul concentrated SABC into 990ul 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, pilot samples, and control (blank) wells on the pre-coated plate respectively and record their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors.
2. **Standards:** Aliquot 100 $\mu\text{l}$  of standard solutions into the standard wells.
3. **Dilution Buffer:** Add 100 $\mu\text{l}$  of Sample dilution buffer into the control (blank) well.
4. **Samples:** Add 100 $\mu\text{l}$  of properly diluted sample into test sample wells.
5. **Incubate:** Seal the plate with a cover and incubate at 37 °C for 90 mins.
6. **Wash:** Remove the cover, aspirate the liquid from the plate and wash plate 2 times with Wash Buffer. **Do NOT let the wells dry completely at any time.**
7. **Biotin-labeled Antibody:** Add 100 $\mu\text{l}$  of Biotin-labelled antibody working solution to the bottom of each well (standard, test sample & zero wells) without touching the side walls.
8. **Incubate:** Seal the plate with a cover and incubate at 37°C for 60 mins.
9. **Wash:** Remove the cover, and wash plate 3 times with Wash buffer. **Let the WashBuffer stay in the wells for 1-2 minutes for each wash.**

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10. **HRP-Streptavidin Conjugate (SABC):** Add 100µl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 mins.
  11. **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.**
  12. **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 13.
  13. **Stop Solution:** Add 50µl of Stop solution into each well and mix thoroughly. The colour changes into yellow immediately.
  14. **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.

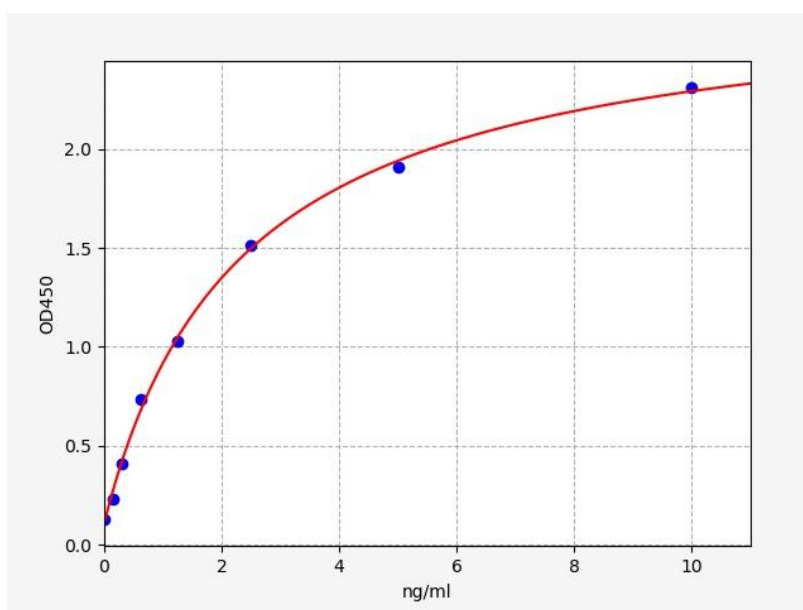
**Note:** If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

## 10. Typical Data & Standard Curve

### Standard Curve

This product has been tested by the Quality Control Department and meets performance specifications mentioned in the manual. (The humidity in the laboratory is 20%-60%, and the temperature is 18°C - 25°C. TMB was balanced to 37°C before color development and incubated at 37°C for 15 minutes in the dark after adding the enzyme label plate holes.) The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.

STD.(ng/ml)	OD-1	OD-2	Average	Corrected
0	0.071	0.073	0.072	0
0.156	0.117	0.121	0.119	0.047
0.312	0.226	0.232	0.229	0.157
0.625	0.414	0.426	0.42	0.348
1.25	0.615	0.633	0.624	0.552
2.5	0.869	0.895	0.882	0.81
5	1.511	1.555	1.533	1.461
10	2.183	2.247	2.215	2.143



## Precision

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

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

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
<b>n</b>	20	20	20	20	20	20
<b>Mean (ng/mL)</b>	0.29	1.2	5.18	0.29	1.24	5.09
<b>Standard Deviation</b>	0.02	0.06	0.24	0.01	0.06	0.24
<b>CV (%)</b>	5.23	4.88	4.68	4.98	5.12	4.69

## Recovery

Matrices listed below were spiked with a certain level of OCT4 and the recovery rates were calculated by comparing the measured value to the expected amount of OCT4 in the samples.

Sample	Recovery Range %	Average %
<b>Serum (n=5)</b>	86-105	93
<b>EDTA Plasma (n=5)</b>	94-102	96
<b>Heparin Plasma (n=5)</b>	88-100	93

## Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of OCT4 and their serial dilutions.

Sample	1:2	1:4	1:8
<b>Serum (n=5)</b>	88-105%	88-96%	95-103%
<b>EDTA Plasma (n=5)</b>	89-101%	83-94%	82-101%
<b>Heparin Plasma (n=5)</b>	87-92%	80-95%	80-100%



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## Precision

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

## Stability

The stability of the OCT4 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.

<b>Standard (n=5)</b>	<b>37°C for 1 month</b>	<b>4°C for 6 months</b>
<b>Average (%)</b>	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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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**



Email: [info@ASSAYGenie.com](mailto:info@ASSAYGenie.com)

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