SinoGeneclon Sinogeneclon Co.,Ltd info@sinogeneclon.com;tech@sinogeneclon.com

# Chicken free thyroxine (FT4) ELISA kit

## A Complete ELISA kit for the detection of Chicken FT4

## (Cat. #SG-80115)

## 【PRODUCT SPECIFICATIONS】

• Assay Principle: Quantitative
• Species:Chicken
• Standard concentration:72pmol/L
• Sensitivity:4pmol/L
• Detection Range:2pmol/L-64pmol/L
•Sample Type: serum , plasma , urine , tissue homogenates , cell culture supernates
• Size: 96 Wells

## 【INTRODUCTION】

This ELISA kit uses Sandwich-ELISA as the method. The Micro-elisa strip plate provided in this kit has been pre-coated with an antibody specific to FT4. Standards or samples are added to the appropriate Micro-elisa strip plate wells and combined to the specific antibody. Then a Horseradish Peroxidase(HRP)-conjugated antibody specific for FT4 is added to each Microelisa strip plate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain FT4 and HRP conjugated FT4 antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of FT4 in the samples by comparing the OD of the samples to the standard curve.

## 【ITEMS SUPPLIED】

| | | |
|---|---|---|
| Assay plate (12 × 8 coated Microwells) | 1 | 2-8˚C |
| Standard | 1×0.5ml | 2-8˚C |
| Standard Diluent | 1×1.5ml | 2-8˚C |
| Stop Solution | 1×6ml | 2-8˚C |
| Sample Diluent | 1×6ml | 2-8˚C |
| Chromogen Solution A | 1×6ml | 2-8˚C |
| Chromogen Solution B | 1×6ml | 2-8˚C |
| HRP-Conjugate Reagent | 1×6ml | 2-8˚C |
| Wash Solution | 1×20ml×30 fold | 2-8˚C |
| Adhesive Strip | 2 | RT |
| Sealed bags | 1 | RT |
| User manual | 1 | RT |

## 【STORAGE CONDITIONS】

The kit is shipped on blue ice. Upon arrival, store kit at 2-8˚C for up to 12 months.

## 【ADDITIONAL ITEMS NEEDED】

. Microplate reader (wavelength: 450nm)
. 37° C incubator
. Automated plate washer (Optional)
. Precision single and multi-channel pipette and disposable tips
. Clean tubes and Eppendorf tubes
. Deionized or distilled water

## 【SAMPLE PREPARATION】

**1. Serum preparation**

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

**2. Plasma preparation**

Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifugated for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifugated again.

**3. Urine samples**

Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

**4. Cell samples**

If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again.

**5. Tissue samples**

Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80℃ for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4 ℃ . Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000rpm. Aliquot the supernatant for ELISA assay and future use.

**Notes:**

1. Sample extraction and ELISA assay should be performed as soon as possible after sample collection. The samples should be extracted according to the relevant literature. If ELISA assay can not be performed immediately, samples can be stored at -20℃. Repeated freeze-thaw cycles should be avoided.

2. Our kits can not be used for samples with NaN3 which can inhibit the activity of HRP.

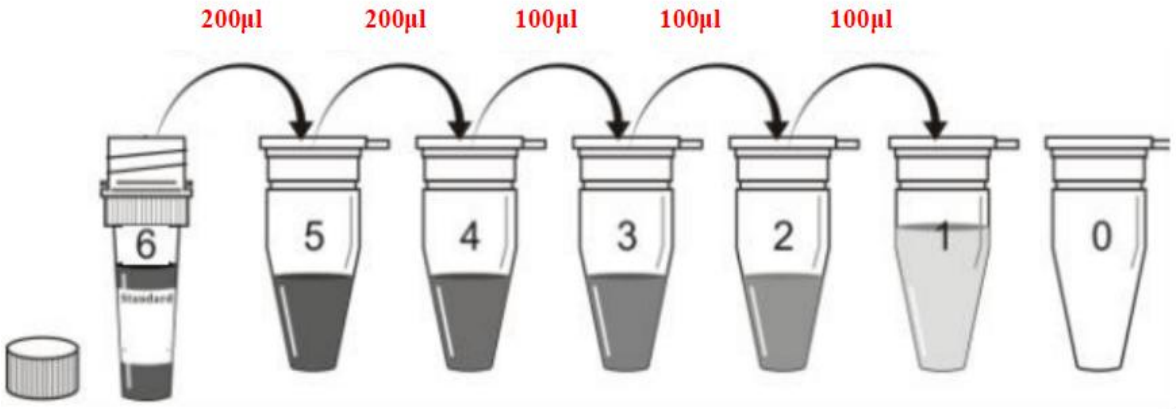
## 【REAGENT PREPARATION】

**1. Prepare wash buffer**

a. Combine 20 ml concentrated wash Solution (whole bottle) with 580 ml DI water

**2. Standard**

Dilute the standard：Pipette 100μl standard diluent in each tube. Pipette 200μl standard in the fifth tube. And take out 200μl from the fifth five tube into the fourth. Pipette 100μl from the fourth tube to the third tube and produce dilution series as below. The undiluted Standard serves as the high standard (72pmol/L ). Sample Diluent serves as the zero standard(blank well).




| Tube | 6 | 5 | 4 | 3 | 2 | 1 | 0 |
|---|---|---|---|---|---|---|---|
| pmol/L | 72 | 48 | 32 | 16 | 8 | 4 | 0 |

## 【PROCEDURE】

**Step 1:** Prepare all reagents, working standards, Blank and samples as directed in the previous sections.

**Step 2:** Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4° C

**Step 3：** Pipette standard 50μl to testing standard well， Pipette Sample diluent 40μl to testing sample well, then add testing sample 10μl (sample final dilution is 5-fold), Pipette sample to wells, don't touch the well wall as far as possible, and mix gently.

**Step 4:** Incubate: Cover with the adhesive strip provided, incubate for 30 min at 37℃.

**Step 5:** Configurate liquid: Dilute wash solution 30-fold with distilled water.

**Step 6:** Washing: Uncover the adhesive strip, discard liquid, pipette washing buffer to every well, still for 30s then drain, repeat 5 times.

**Step 7:** Add enzyme: Pipette HRP-Conjugate reagent 50μl to each well, except blank well.

**Step 8:** Incubate: Operation with 4

**Step 9:** Washing: Operation with 6.

**Step10:** Color: Pipette Chromogen Solution A 50ul and Chromogen Solution B 50ul to each well, avoid the light preservation for 15 min at 37℃.

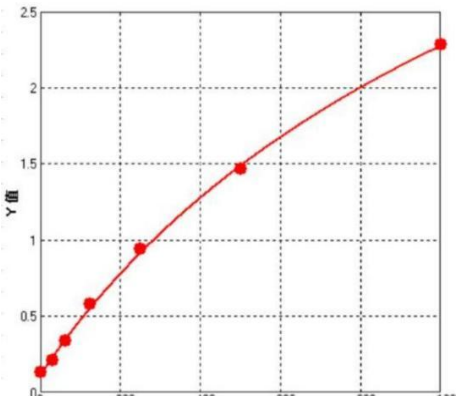
**Step 11:** Stop the reaction: Pipette Stop Solution 50μl to each well, stop the reaction (the blue change to yellow).

**Step 12:** Calculate: take blank well as zero. Read absorbance at 450nm after pipette Stop Solution within 15min.

## 【Calculation of result】

Take the standard concentration as the horizontal, the OD value for the vertical ,draw the standard curve on graph paper, Findout the corresponding concentration according to the sample OD value by the Sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with the standard concentration and the OD value, with thesample OD value in the equation, calculate the sample concentration, multiplied by the dilution factor, the result is the sample actual concentration.

Graphical Representation as following:




## 【Expiration】

Twelve months [see label on the outer box for the specific date]

## 【Precision】

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Chicken FT4 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Chicken FT4 were tested on 3 different plates, 8 replicates in each plate.

CV(%) = SD/mean X 100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

## 【Attention】

1: The kit takes out from the refrigeration should be balanced 15-30 minutes in the room temperature, if the coated ELISA plates have not been used up after opening, the plate should be stored in sealed bag.

2: Washing buffer will Crystallization separation, it can be heated in water to dissolve.

3: Pipette sample with pipettors each step, and proofread its accuracy frequently to avoid the experimental error. Pipette sample within 5 min, if the number of sample is big, recommend using multichannel pipettor.

4: If the testing material concentration is excessively high (The sample OD is higher than the first standard well) ,please dilute the sample (n-fold).

5: Adhesive Strip only limits the disposable use to avoid cross-contamination.

6: The substrate should evade the light to be preserved.

7: Please refer to the user instruction strictly, the test result determination must take the microtiter plate reader as a standard.

8: The preparation of samples and all the reagents should refer to infective material process.

9: Do not mix reagents with those from other lots.

## 【Washing method】

Manually washing method: shake away the remained liquid in the enzyme plates; place some bibulous papers on the test-bed, and flap the plates on the upside down strongly. Inject at least 0.35ml after-dilution washing solution into the well, and marinate 1~2 minutes. Repeat this process according to your requirements.

Automatic washing method: if there is automatic washing machine, it should only be used in the test when you are quite familiar with its function and performance .

## 【Troubleshooting】

| Possible Case | Solution |
|---|---|
| **High Background** | |
| *Improper washing<br><br>*Substrate was contaminated<br><br>*Non-specific binding of antibody<br><br>*Plate are not be sealing incompletely<br><br>*Incorrect incubation temperature<br><br>*Substrate exposed to light prior to use<br><br>*Contaminated wash buffer | *Increasing duration of soaking steps<br><br>*Replace. Substrate should be clean and avoid crossed contamination by using the sealer<br><br>*Replace another purified antibody or blocking buffer<br><br>*Make sure to follow the instruction strictly<br><br>*Incubate at room temperature<br><br>*Keep substrate in a dark place<br><br>*Use a clean buffers and sterile filter |
| **Weak Signal** | |
| *Improper washing<br><br>*Incorrect incubation temperature<br><br>*Antibody are not enough<br><br>*Reagent are contaminated<br><br>Pipette are not clean | *Increasing duration of soaking steps<br><br>*Incubate at room temperature<br><br>*Increase the concentration of the antibody<br><br>*Use new one Pipette should be clean |
| **No Signal** | |
| *Reagent are contaminated<br><br>*Sample prepared incorrectly<br><br>*Antibody are not enough<br><br>*Wash buffer contains sodium azide HRP was not added | *Use new one<br><br>*Make sure the sample workable/dilution<br><br>*Increase the antibody concentration<br><br>*Use a new wash buffer and avoid sodium azide in it<br>*dd HRP according to the instruction |
| **Poor Precision** | |
| *Imprecise/ inaccurate pipetting<br><br>*Incomplete washing of the wells | *Check/ calibrate pipettes<br><br>*Make sure wells are washed adequately by filling the wells with wash buffer and all residual antibody solutions crossed well before washing. |

For technical support contact information,tech@sinogeneclon.com

SinoGeneclon

**www.sinogeneclon.com**