# Free Triiodothyronine (fT3)

## ENZYME IMMUNOASSAY TEST KIT

**Enzyme Linked Immunosorbent Assay (ELISA) for Quantitative Determination of Free Triiodothyronine (fT3) in Human Serum**

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

### INTENDED USE

**fT3** Competitive ELISA test is intended for the quantitative determination of Free Triiodothyronine (fT3) in human serum. For In Vitro Diagnostic Use only.

### INTRODUCTION

T3 circulated in the blood as an equilibrium mixture of free and protein bound hormone. T3 is bound to thyroxine binding globulin (TBG), prealbumin and albumin. The binding of these proteins is such that only 0.2%-0.4% of the total T3 is present in solution as unbound or free T3 (fT3). This free fraction represents the physiologically active thyroid hormone and is three to four times more potent than T4. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alters, the total triiodothyronine level changes so that the free triiodothyronine concentration remains constant. Thus, measurements of free triiodothyronine concentrations correlate more reliably with clinical status than total triiodothyronine levels.

### PRINCIPLE OF THE ASSAY

**fT3** Quantitative Test Kit is a Competitive enzyme-linked immunosorbent assay. In this test, a certain amount of anti- T3 antibody is coated on microtiter wells. A measured amount of patient serum and a constant amount of T3 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, free T3 in the sample reacts with the anti-T3 antibody and conjugated T3 compete for the limited binding sites. After incubation, the wells are washed and bound enzyme is detected by adding substrate. The reaction is stopped after specified time with stop solution and absorbance is determined for each well using an ELISA reader. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled fT3 in the sample. By reference to a series of fT3 standards assayed in the same way, the concentration of fT3 in the unknown sample is quantified.

### MATERIALS AND COMPONENTS

***Materials provided with the test kits:***

1. Coated Microwells: Microwells coated with Anti-T3 antibody.
2. fT3 HRPO Conjugate Diluent.
3. fT3 HRPO Enzyme Conjugate concentrate (20X).
4. TMB Substrate. Ready to use.
5. Stop Solution. Ready to use.
6. fT3 Standard set of 6 Standards labeled as A to F in liquid form. Ready to use. For Standard Concentrations refer vial label.
7. Wash Buffer Concentrate (20X).
8. Control Set.
9. Pack Insert.
10. Plate Sealer.
11. Protocol Sheet.
12. Microwell holder.

***Materials required but not provided***

1. Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
2. Disposable pipette tips
3. Distilled water
4. Disposable Gloves
5. ELISA reader
6. ELISA washer

### STORAGE AND STABILITY

1. **fT3** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the dessicant has changed from blue to pink at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable upto one week when stored at 2-8°C.

### SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, Specimens can be stored up to 48 hours at 2-8°C for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

### PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens, standards and controls should be considered potentially Infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

### REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
3. Dilute Enzyme Conjugate with Conjugate Diluent according to the requirement as shown below. Prepare a fresh dilution for each assay.

| No. of Strips | 0.5 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Enzyme Conjugate(µl) | 25 | 50 | 100 | 125 | 175 | 200 | 250 | 300 | 350 | 375 | 400 | 450 | 500 |
| Conjugate Diluent (µl) | 500 | 1000 | 2000 | 2500 | 3500 | 4000 | 5000 | 6000 | 7000 | 7500 | 8000 | 9000 | 10000 |

### TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **50 µl** of standards, controls and sera into the appropriate wells.
2. Dispense **100 µl** of diluted Conjugate into each well. Incubate at room temperature (18-25 °C) for **45 minutes.**
3. After incubation, empty the microtitre wells and wash the plate **5 times** with **350 µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
4. Dispense **100 µl** of TMB Substrate into each well. Incubate at room temperature (18-25 °C), in the dark, for **20 minutes**.
5. Stop the reaction by adding **100 µl** of Stop Solution to each well. Gently mix for **10 seconds** until the blue color completely changes to yellow.
6. Read the optical density at 450/630 nm with a microtiter plate reader within **15 minutes**.

Add **50 µl** standards/controls/samples into the respective Microwells

⬇

Add **100 µl** diluted Conjugate in each well

⬇

Apply plate sealer and incubate for **45 minutes** at **18-25°C**

⬇

Wash Microwells **5 times** with **350 µl** of diluted Wash Buffer

⬇

Add **100 µl** Substrate in each well

⬇

Incubate for **20 Minutes** at **18-25°C in dark**

⬇

Add **100 µl** Stop Solution in each well

⬇

Read results @450nm (Ref.600-700nm) within **15 minutes**

### CALCULATION OF RESULTS

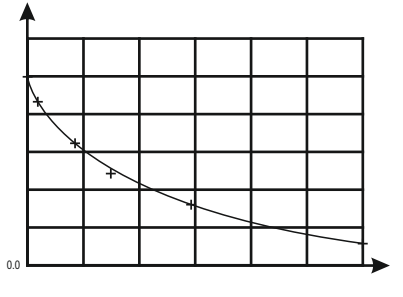
Construct a Standard curve by plotting the absorbance obtained from each reference standards against its concentrations in pg/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of fT3 in pg/ml from the Standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

**Example of Standard curve**

Results of a typical standard run with optical density reading at 450nm (ref 600-700nm) shown in the Y axis against fT3 concentrations shown in the X axis.

**Suggest: Use 4-Parameter Standard curve to calculate sample values.**

| fT3 Values (pg/ml) | Absorbance (450nm) |
|---|---|
| A | 1.9550 |
| B | 1.6660 |
| C | 1.4799 |
| D | 0.8819 |
| E | 0.2428 |
| F | 0.0572 |



This Standard curve is for the purpose of illustration only and should not be used to calculate samples. Each user should obtain his or her own Standard curve and data.

**Expected Ranges of values**

A study of euthyroid adult population was undertaken to determine expected values for the fT3 EIA Test System. The mean (X) values, standard deviations (SD) and expected ranges ± 3 SD are presented below:

Expected Values for the Free T3 EIA Test System (in pg/ml)

| | Adult (107 specimens) |
|---|---|
| Mean (X) | 2.86 |
| Standard Deviation (SD) | 0.45 |
| Expected Ranges (± 3SD) | 1.52 – 4.21 |

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

The minimal detectable concentration of fT3 by this assay is estimated to be 1.475 pg/ml.

**PERFORMANCE CHARACTERISTICS**

**A) Internal Evaluation:**

1. Accuracy: In an internal study **fT3** was evaluated against commercially available licensed kit with 90 random clinical samples & **fT3** has demonstrated 100% clinical correlation with the commercially available licensed kit.
2. Precision: **fT3** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

| Controls | No. of testings | Mean Control values with **fT3** | Coefficient of Variation (CV) |
|---|---|---|---|
| Level 1 | 10 | 2.71 | 5.19 |
| Level 2 | 10 | 5.37 | 6.15 |
| Level 3 | 10 | 7.18 | 6.65 |

**B) External Evaluation:**

**fT3** ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **fT3** ELISA has demonstrated 100% correlation with the reference method.

*Data file: Orchid Biomedical Systems (P) Ltd.

**IMPORTANT NOTE**

1. The fT3 assay is a temperature sensitive assay. The best temperature condition for this assay is from 18°C to 25°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of standards, samples and controls is not mandatory but may provide information on reproducibility & application errors.

**LIMITATIONS OF THE ASSAY**

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

**BIBLIOGRAPHY**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8. Data on file: Orchid Biomedical Systems (P) Ltd.


**SYMBOL KEYS**

| | |
|---|---|
| Temperature Limitation | Consult Instructions for use |
| Manufacturer | *In vitro* Diagnostic Medical Device |
| Use by | Catalogue Number |
| Date of Manufacture | Batch Number / Lot Number |
| This side up | Contains sufficient for <n> tests |
| Do not reuse | |


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