



**Thyronine(T4)  
ELISA Test Kit  
(Serum/Plasma)  
Package Insert**

*For in vitro diagnostic and professional use only.*

**PRODUCT NAME**

T4 ELISA Test Kit (Serum/Plasma)

**INTENDED USE**

This kit is a quantitative detection of human serum/plasma of thyronine (T4). The reagent is suitable for clinical screening and diagnosis of thyronine (T4) in human serum/plasma.

T4 ELISA Kit is intended for professional use as an aid in the diagnosis of thyroid disorder. T4 values should not be the only factor in the determination of thyroid status, and all other available clinical and epidemiological information should be considered for an accurate diagnosis.

Recommended for use:

Aid in diagnosis of hyper- and hypothyroidism

Distinguish between different thyroid disorder

Aid in diagnose of pituitary disorder and female infertility

Monitor patients undergoing treatment for thyroid disorder, pituitary disease, and thyroid cancer

Quantitative measurement of the more stable T4/T3 levels is believed to be more accurate in most cases. In a normal thyroid testing algorithm, T4 tests are usually ordered after TSH levels are measured in the blood.

If T4 levels are abnormal, a diagnosis can be made depending on the combination of TSH and T4 levels. For example, low TSH and high T4 levels normally indicate hyperthyroidism, but high TSH and low T4 levels may indicate hypothyroidism

**PRINCIPLE**

When thyroid function decrease, T4 value will go down. And T4 value decrease is before T3 value decrease. So T4 detection along with TSH detection is good for thyroid decrease detection. Thyroid absence or congenital hypoplasia, thyroid removed, any of them will cause serum T4 decrease.

When hypothyroidism appears, it includes primary, secondary, triple hyperthyroidism, hyperfunctional adenoma, autonomic nodules, T4 hyperthyroidism, etc, all of them will cause serum T4 increase.

The system of the T4 ELISA test is founded on the solid phase, one-step incubation competitive principle. When T4 is present, it competes with T4 antigen conjugated to horseradish peroxidase (HRP-Conjugate) for a fixed amount of purified anti-T4 pre-coated in the wells. In the course of washing, any unbound HRP-Conjugate is removed. After chromogen solutions A and B are added into the wells and incubation, the result will be calculated with enzyme microplate reader after stop solution is added.

**MAIN COMPONENTS**

Materials provided with the kit:

Components	96T		480T	
coated Microtiter plate	1 bag	12*8	5 bags	12*8
Conjugate	1 vial	6.5 mL	5 vials	6.5 mL

Wash Buffer (40X)	1 vial	20 mL	5 vials	20 mL
Substrate A	1 vial	7 mL	5 vials	7 mL
Substrate B	1 vial	7 mL	5 vials	7 mL
Blockers	1 vial	6.5 mL	5 vials	6.5 mL
Stop Solution	1 vial	6 mL	5 vials	6 mL
Control S0 (0 ng/mL)	1 vial	0.5 mL	5 vials	0.5 mL
Control S1 (20 ng/mL)	1 vial	0.5 mL	5 vials	0.5 mL
Control S2 (40 ng/mL)	1 vial	0.5 mL	5 vials	0.5 mL
Control S3 (80 ng/mL)	1 vial	0.5 mL	5 vials	0.5 mL
Control S4 (160 ng/mL)	1 vial	0.5 mL	5 vials	0.5 mL
Control S5 (320 ng/mL)	1 vial	0.5 mL	5 vials	0.5 mL
Closure plate membrane	2 sheets		10 sheets	

**Note: different batches of reagent kit, and different component cannot be exchanged for use.**

**Once open, stable for 3 months at 2-8°C.**

**SAMPLE REQUIREMENT**

1. **Specimen Collection:** No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum must be separated from the clot as early as possible to avoid hemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms.

2. **Highly lipaemic, icteric, or hemolytic specimens should not be used** as they can give false results in the assay. **Do not heat inactivate specimens.** This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.

3. T4 ELISA is intended ONLY for testing of individual serum samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

4. Transportation and Storage: Store specimens at 2-8°C. Specimens not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

**TEST PROCEDURE**

1. All reagents should be allowed to reach room temperature for 15 minutes before use.

2. Dilute the wash buffer at the rate of 1:40 dilution with distilled water before use.

3. The sample should be corresponding to the number of micro plate, each plate should be provided with sample and reference material wells(The number of well is determined by sample and reference material).

**Note: Use a separate disposal pipette tip for each specimen, each control as to avoid cross contamination.**

4. Add 50µL sample in the corresponding well, add 50µL each control S0, S1, S2, S3, S4, S5 respectively to corresponding well respectively.

5. Add 50µL blockers into each well.

6. Respectively adding 50µL conjugate into each well. Shake gently to mix for 30s(An important step). Incubate at 37 °C for 60 minutes with the sealing plate membrane sealing the plate.

7. At the end of the incubation, remove and discard the plate cover. Take out, add wash buffer to

each well for 10 seconds. Repeat 5 times. After the final washing cycle, turn the plate over onto blotting paper or clean towel, and tap it to remove any remainders.

8. Add Substrate A (50µL) and Substrate B (50µL) into each well. Incubate at 37 °C for 10 minutes with the sealing plate membrane sealing the plate.

9. Add 50µL Stop Solution to each well. And read the absorbance value at 450nm/630nm with microplate reader. Then calculate the concentration and evaluate the results.

#### DATA HANDING

Fit Type: 4 parameters Logistic

Coordinate Choice: X-Y

#### REFERENCE VALUE (OR RANGE)

T4 normal concentration (Just for reference): 50~140ng/mL. And it is calculated by normal distribution method.

This normal concentration should be built by themselves during the long time practice for each test room.

#### INTERPRETATION OF RESULTS

Chronometry: Read the sample's optical density (OD) at 450nm with a microplate reader.

The detection limit is 5.0 ng/mL. And the detection range is 5.0~320.0 ng/mL. If the determination value is higher or lower than normal range, it means there is an abnormal result. The final result should be diagnosed with clinical symptom and other diagnose method.

Some result out of this detect range calculated through reference curve extension can not be used as accurate quantitative result. The result of high concentration sample out of detection range should be detected after diluted.

#### LIMITATIONS

1. It is no insignificance for positive results must be confirmed with another available method, and it should be interpreted in conjunction with the patient clinical information.

2. This reagent is only used for the detection of human serum samples.

1. Dosage reflected the curve linear

Correlation coefficient of the standard curve ( $|r|$ ) should be  $\geq 0.9900$  at the range of 5.0ng/mL~32.0ng/mL.

2. The lowest limit: T4 is not higher than 5.0ng/mL

3. Precision

Intra-assay

Manual Operation : CV% $\leq$ 15%; Automatic System: CV% $\leq$ 8.0%

Inter-assay: CV% $\leq$ 20%

4. Accuracy

Test it with TSH national standards as contrast, and (the test result of reference material/the test result of the marked value) is at the range of 0.900~1.100.

5. Specificity

The test result is not higher than 15.0ng/mL with this test kit for TT3 which concentration is not lower than 500ng/mL

The test result is not higher than 15.0ng/mL with this test kit for rT3 which concentration is not lower than 50ng/mL

#### ATTENTIONS

1. Do not exchange reagents from different lots or use reagents from other commercially available kits.

The components of the kit are precisely matched for optimal performance of the tests.

2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.

3. Allow the reagents and specimens to reach room temperature before use. Shake reagent gently before use.

4. Concentrated washing liquid will produce crystal at room temperature, should be diluted completely before use.

5. Please put the unused plate back into the bag, and store at 2~8°C.

6. Operate strictly according to the instruction, control of reaction time and temperature strictly.

7. Never reuse microplate sealing membrane. If the external of the microplate contact with water when warm bath, results will be better.

8. Use sufficient volume of washing liquid in the washing steps. Fail to do so may cause color deepening.

9. T4 reference material is limited to use in this ELISA kit but sales alone.

#### STORAGE AND EXPIRY

1. Store at 2-8°C. DO NOT FREEZE.

2. Once open, stable for 3 months at 2-8°C. Other liquid components have the same validity period with the reagent box.

#### BIBLIOGRAPHY

[1] Pharmacopoeia of the People's Republic of China.

[2] Requirements for Biologics of the People's Republic of China.

#### MANUFACTURER

#### BIOGENIX INC. PVT. LTD.









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#### INSTRUCTIONS OF SYMBOL

	Consult instruction for use		Keep dry
	Store between	<b>LOT</b>	Batch number
	Do not re-use	<b>IVD</b>	IVD product
	Manufacturer		Date of manufacture
	Expire date		Contains sufficient for <n> tests