# General Thyroxine (T4) ELISA Kit

## Cat #: orb1974531 (manual)

### INTENDED USE

The kit is used for the quantitative determination of Thyroxine (T4) content in samples such as serum, plasma, and other biological fluids.

### ASSAY PRINCIPLE

This kit employs the competitive ELISA principle. The microplate wells are pre-coated with purified thyroxine (T4) antibody. Standards and test samples are sequentially added to the coated wells, together with biotin-labeled antigen. The test antigen and the biotin-labeled antigen compete for binding to the specific antibody. After incubation, unbound materials are removed by washing. Horseradish peroxidase (HRP)-labeled avidin is then added to the wells. Biotin and avidin form a strong non-covalent interaction. Following thorough washing, the TMB substrate is added for color development. TMB is converted to a blue color under the catalytic action of HRP and ultimately turns yellow after the addition of an acid solution. The higher the concentration of the test antigen, the more the binding between the labeled antigen and the antibody is inhibited, resulting in lighter color development. The color intensity is positively correlated with the amount of the enzyme and negatively correlated with the concentration of the test sample.

### REAGENTS AND MATERIALS PROVIDED

| Reagents | Quantity (96T) | Storage |
|---|---|---|
| Pre-coated, ready to use 96-well strip plate | 8×12 | -20°C |
| Standard | 2 | -20°C |
| Diluent | 1×45 mL | -20°C |
| Detection Reagent A | 1×70µL | -20°C |
| Detection Reagent B | 1×120µL | -20°C |
| Wash Buffer (30 ×) | 1×20mL | 2-8°C |
| TMB substrate | 1×9 mL | 2-8°C, protect from light |
| Stop Solution | 1×6 mL | 2-8°C |
| Plate Sealer | 4 pieces | |
| Manual | 1 copy | |

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader (wavelength 450 nm)
2. High-precision adjustable pipettes (calibrated) and tips: 0.5–10, 2–20, 20–200, 200–1000 µL. A multichannel pipette is recommended when processing a large number of samples per assay.
3. Automatic plate washer or wash bottle
4. 37°C incubator
5. Double-distilled water or deionized water
6. Graph paper
7. Graduated cylinder

**Notes:**

1. Store the kit at 2-8°C and -20°C. It is recommended to discard any reconstituted but unused standard. Do not mix components from different lots or sources. Use the product before the expiration date.

2. Upon removal from low temperature, concentrated wash buffer may develop crystals. During dilution, warm the buffer in a water bath to dissolve the crystals; this does not affect performance.

3. Use calibrated pipettes for all addition steps to avoid errors. It is recommended to complete each addition step within 5 minutes. A multichannel pipette is recommended when processing a large number of samples.

4. To avoid cross-contamination, change tips promptly when adding different concentrations of standards, different samples, or different reagents. The plate sealer is disposable.

5. Store the TMB substrate protected from light.

6. After opening, place the microplate in a sealed bag with desiccant and store at -20°C, protected from moisture.

## SAMPLE COLLECTION AND STORAGE

1. Serum: Allow blood samples to clot naturally at room temperature for 60–120 minutes. Centrifuge at 1000g for 20 minutes. Collect the supernatant. If precipitate forms during storage, centrifuge again. Avoid repeated freeze-thaw cycles.

2. Plasma: Choose EDTA or citrate as an anticoagulant according to the sample requirements. Centrifuge at 1000g for approximately 20 minutes and collect the supernatant. If precipitate forms during storage, centrifuge again.

3. Other biological samples: To detect secreted components, collect samples in sterile tubes. Centrifuge at 1000g for approximately 20 minutes and collect the supernatant.

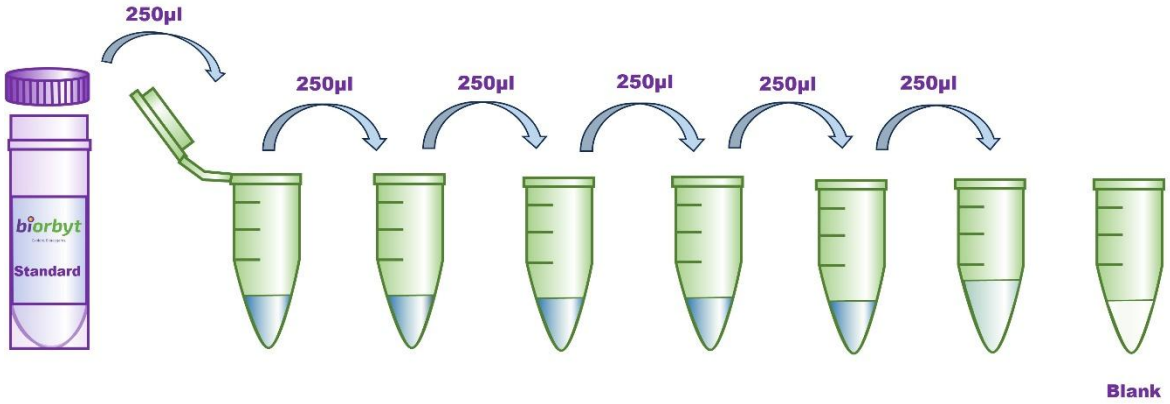
4. If samples cannot be tested immediately, aliquot the minimum required amount and store at -20°C to -70°C. Avoid repeated freeze-thaw cycles. Avoid using hemolyzed or hyperlipidemic samples whenever possible.

## REAGENT PREPARATION

**1. Reagent equilibration:** Allow the kit and test samples to equilibrate to room temperature before the experiment.

**2. Wash buffer:** Dilute the concentrated wash buffer to 1X working solution with double-distilled or deionized water according to its concentration factor.

**3. Standard:**
Add 1 mL of Standard/Sample Diluent (S1) to the lyophilized standard. Let stand for 15 minutes to allow complete dissolution, then mix gently (concentration: 80 ng/mL). Prepare 6 polypropylene tubes, add 250 µL of diluent to each tube, and perform 2-fold serial dilutions to obtain the following concentrations: 40, 20, 10, 5, 2.5, and 1.25 ng/mL. The 80 ng/mL standard serves as the highest point of the standard curve, and the diluent is used as the zero point (0 ng/mL). Discard any unused reconstituted standard stock solution (80 ng/mL).




| Tube | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 |
|---|---|---|---|---|---|---|---|---|
| ng/mL | 80 | 40 | 20 | 10 | 5 | 2.5 | 1.25 | 0 |

4. **Detection Reagent A and Detection Reagent B Working Solution:** Before use, gently flick the vials or centrifuge briefly to bring down any liquid adhering to the tube walls or cap. Immediately prior to use, dilute each solution 1:100 with **Diluent** (e.g., 10 µL of Detection Reagent A or B in 990 µL of Diluent) and mix thoroughly. Prepare the required amount based on the pre-calculated total volume needed for each experiment (100 µL/well). When preparing, make an additional 0.1–0.2 mL more than the calculated volume.

## ASSAY PROCEDURE

1. **Sample Addition:** Based on the required amount for the assay, take out the appropriate pre-coated plate strips. Add 50 µL per well of the prepared standards, standard zero (blank), and test samples to the bottom of the wells.
2. **Addition of Detection Reagent A** (prepared fresh before use)**:** Immediately add 50 µL of Detection Reagent A working solution to each well.
3. **Incubation:** Seal the plate with plate sealer and incubate at 37°C for 60 minutes.
4. **Washing:** Carefully remove the plate sealer, discard the liquid, and tap the plate dry. Fill each well with washing buffer (350 µL), let stand for 1–2 minutes, then discard. Repeat this process 3 times then blot dry on absorbent paper.
5. **Addition of Detection Reagent B** (prepared fresh before use)**:** Add 100 µL of Detection Reagent B working solution to each well.

6. **Incubation:** Seal the plate with adhesive film and incubate at 37°C for 60 minutes.
7. **Washing:** Repeat the washing procedure as described above (Step 4), wash the plate 5 times.
8. **Color Development:** Add 90 µL of substrate solution to each well. Seal the plate and incubate at 37°C for 15–25 minutes for color development.
9. **Stopping the Reaction:** Add 50 µL of stop solution to each well (the color will change from blue to yellow immediately).
10. **Measurement:** Measure the absorbance (OD value) of each well at 450 nm using a microplate reader. The measurement should be completed within 5 minutes after adding the stop solution.

## CALCULATION OF RESULTS

1. The OD value for each standard and sample should be taken as the average of duplicate (or replicate) wells.

2. Using computer software, generate a standard curve by plotting the absorbance (OD) values on the Y-axis against the corresponding T4 standard concentrations on the X-axis. The T4 concentration in samples can be determined by interpolating their OD values from the standard curve. If the samples have been diluted, the calculated concentration must be multiplied by the appropriate dilution factor.

## PERFORMANCE

**DETECTION RANGE**
1.25 ng/ml -80ng/ml

**SENSITIVITY**
0.63 ng/ml

**PRECISION**
CV (%) = SD/meanX100
Intra-Assay: CV<10% Inter-Assay: CV<10%

## PRECAUTION

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this reagent.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558