T3
ELISA Test for the Quantitative Determination of Total Triiodothyronine (T3) in Human Serum or Plasma

Package Size

| REP | 54010 | 96 Tests Complete Test Kit |

Intended Use

Triiodothyronine (T3) is a hormone synthesised and stored in the thyroid gland. More than 99% of T3 in the blood is bound reversibly to plasma proteins. The concentration of T3 is much lower than that of T4, but its metabolic potency is much greater. T3 determination is an important tool in thyroid disease diagnosis. Its measurement has uncovered a variant of hyperthyroidism where thyrotoxic patients present elevated T3 values with normal T4 values (T3-hyperthyroidism). An increase in T3 without an increase in T4 is frequently a forerunner of recurrent thyrotoxicosis in previously treated patients. The clinical significance of T3 is also evident in patients in whom euthyroidism is attributable only to normal T3, though their T4 values are subnormal. T3 determination is also useful in monitoring both patients under treatment for hyperthyroidism and patients who have discontinued antithyroid drug therapy. It is especially valuable in distinguishing between euthyroid and hyperthyroid subjects. In addition to hyperthyroidism, T3 levels increase during pregnancy, oral contraception or estrogen treatment, paralleling TBG (Thyroxine Binding Globulin) increases in a manner analogous to T4. Likewise, a decrease in TBG concentration decreases T3 concentration. These changes in the T3 level, however, are not a true reflection of thyroid status. Best diagnostic information about the thyrotoxic state in such situations can be obtained by the TRH test.

Principle

- Competitive EIA -

The T3 ELISA is based on the principle of competitive binding between T3 in a test specimen and T3-peroxidase conjugate for a limited number of binding sites on the anti-T3 (sheep) coated well. Thus the amount of T3-peroxidase conjugate bound to the well is inversely proportional to the concentration of T3 in the specimen. After incubation of specimen and T3-peroxidase conjugate unconjugated enzyme conjugate is removed in the equilibrium state by washing. TMB/substrate solution is added (step 2), and a blue colour develops. The intensity of this colour, which changes to yellow after stopping the reaction, is inversely proportional to the amount of T3 in the specimen.

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN’s HUMAREADER or ELISISY line). Specimen's concentration is extrapolated from a dose response curve generated by utilising serum calibrators of known antigen concentrations.

Reagents and Contents

| MB | 12 | Microtiter Strips (in 1 strip holder) |
| CAL | A - F | Calibrators (white cap) |
| 6x2.0 ml | Ready to use, in human serum |
| T3 level: | 0 (A), 0.50 (B), 1.00 (C), 2.50 (D), 5.00 (E) and 7.50 (F) ng/ml |
| CON | 1.5 ml | Enzyme-antigen conjugate (white cap) |
| pH 7.45 ± 0.1 | T3-HRP-conjugate, coloured yellow |
| in a protein stabilising matrix | 1 % |
| DR | 13 ml | Conjugate buffer (white cap) |
| pH 7.42 ± 0.1 | Phosphate buffer, coloured red |
| WS | 20 ml | Wash Solution (black cap) |
| pH 8.8 ± 0.4 | Concentrate for ca. 1000 ml |
| MOPS buffered saline | 5 mmol/l |
| BA | 7.0 ml | Substrate Reagent A (yellow cap) |
| pH 3.5 ± 0.1 | 33, 5,5'-tetramethylbenzidine (TMB) |
| 4 mmol/l | Sodium acetate buffer |
| 0.05 mol/l | |
| BR | 7.0 ml | Substrate Reagent B (blue cap) |
| pH 4.5 ± 0.1 | Urea hydrogen peroxide |
| 10 mmol/l | Sodium acetate buffer |
| 0.05 mol/l | |
| STP | 7.5 ml | Stop solution (red cap) |
| 0.5 mol/l | Sulphuric acid |
| 1 | Adhesive strip |

Preservatives: Total concentration < 0.04%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and CAL should be handled as potentially infectious. CAL have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or CAL should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use. Reagents not in use should always be stored at 2...8°C.

Working conjugate solution [WCON]

Dilute CON 1 + 10 with C-DIL e.g. dilute 160 μl CON with 1.6 ml C-DIL for 16 wells.

Stability: 24 h at 2...8°C.

Working Wash Solution [WASH]

- faint turbidity, which may appear in the concentrate [WS], will completely dissolve on dilution.
- dilute [WS] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.

Substrate Working Solution [SUB]

- for longer periods of usage: prepare needed amount by mixing equal portions of [SA] and [SS]. Use only a clean plastic vial previously rinsed with deionised water.
- for use within 30 days: pour contents of the vial [SA] in vial [SS] mix and store at 2...8°C.
- handle [SS] carefully and avoid contamination! Do not use, if it looks blue!
- Store protected from bright light.

Stability: 30 days at 2...8°C

Specimen

Serum or plasma (EDTA, Heparin). Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 48 hours at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record CAL specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MB] - select the required number and place firmly in the holder.

P5: Run duplicates for CAL controls and specimens. Pipe them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.
W2: In case of automatic washers fill and prime with The wash procedure is critical. Insufficient washing will result in
Subsequently wash strips 3 times. Ensure the washer fills all wells
aspirate off after 30 sec. soak time and repeat washing twice.
W1: Remove adhesive strips, aspirate off the contents, add
completely and aspirates off efficiently after 30 sec. (remaining
without spilling the solutions to ensure thorough mixing. If available
mix on a plate shaker.

Wash Procedure
The wash procedure is critical. Insufficient washing will result in
poor precision or falsely high absorbance.
W1: Remove adhesive strips, aspirate off the contents, add WASH
aspirate off after 30 sec. soak time and repeat washing twice.
W2: In case of automatic washers fill and prime with WASH.
Subsequently wash strips 3 times. Ensure the washer fills all wells
completely and aspirates off efficiently after 30 sec. (remaining
liquid: < 15 µl).
W3 After washing, remove remaining liquid by tapping the plate
upside down on tissue paper.

Pipetting Scheme
Reagents and specimens should be at room temperature before use.

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Well [µl]</th>
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</thead>
<tbody>
<tr>
<td>A1...D2</td>
<td>[CAL] A-F; in duplicate</td>
</tr>
<tr>
<td></td>
<td>Specimens, Controls; in duplicate</td>
</tr>
<tr>
<td>[WCON]</td>
<td>100</td>
</tr>
</tbody>
</table>

Mix and cover [MC] with Adhesive Strip
Incubate 60 min. at 20...25°C
Wash 3 times as described (see W1 - W3)
[WASH]

<table>
<thead>
<tr>
<th>Step 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>[SUB]</td>
<td>100</td>
</tr>
<tr>
<td>[STOP]</td>
<td>50</td>
</tr>
</tbody>
</table>

Measure the absorbance at 450 nm as soon as possible or
within 10 min. after terminating of reaction, using a reference
wavelength of 630-690 nm (if available).

Validation of the Test
The test results are valid provided the following criteria are met:

Maximum absorbance (calibrator 2A) O.D. ≥ 1.5
T3 concentration at 80% maximum absorbance = 0.60 ± 0.20 ng/ml
T3 concentration at 50% maximum absorbance = 1.85 ± 0.35 ng/ml
T3 concentration at 20% maximum absorbance = 5.50 ± 1.25 ng/ml

Calculation
A dose response curve is used to interpolate the concentration of triiodothyronine in unknown specimens.
1. [CAL]: Plot the absorbance for each duplicate versus the corresponding T3 concentration in ng/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).
2. Draw the best-fit curve through the plotted points.
3. To determine the concentration of T3 for an unknown sample (S), locate the average absorbance of the duplicates on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/dl) from the horizontal axis of the graph.

Interpretation of Results
Total serum triiodothyronine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, TBG concentration, and the binding of triiodothyronine to TBG3
Thus, total triiodothyronine concentration alone is not sufficient to assess the clinical status.
Total serum triiodothyronine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A decrease in T3 values is found with protein-wasting diseases, certain liver diseases and administration of hormones and drugs3.

Expected Values
Results from a study with euthyroid subjects:

| Mean (X) | 1.36 ng/ml |
| Standard Deviation (S.D.) | 0.33 ng/ml |
| Expected Ranges (± 2 S.D.) | 0.69 - 2.02 ng/ml |

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory as the T3 levels are much influenced by geographical and dietary factors.

Performance Characteristics
The T3 ELISA test has a analytic sensitivity of about 0.05 ng/ml T3. Specimens with T3 concentrations above 7.5 ng/ml may be diluted with CAB A and reassayed. To obtain the sample’s concentration multiply by the dilution factor.

Typical performance data can be found in the Verification Report, accessible via www.human.de/data/gb/vr/el-t3.pdf or www.human-de.com/data/gb/vr/el-t3.pdf

Note
The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*) This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.

References
3. Young, D.S. et al., Effects of Drugs on Clinical Laboratory Tests, Clinical Chemistry 21, 3660 (1975)
4. Sterling, L., Diagnosis and Treatment of Thyroid Disease, Cleveland CRC Press, p. 19 - 51 (1975)