FREND™ Total T3

Total Triiodothyronine

Intended use

The FREND™ Total T3 Test System, is a rapid indirect competitive fluorescence immunoassay for the quantitative determination of Total Triiodothyronine (TT3) in human serum and lithium heparinized plasma specimens using the FREND™ System. Measurements of TT3 are used in the diagnosis of thyroid disorders. The FREND™ Total T3 test system is intended for use in clinical laboratories.

Summary and explanation of test

The main thyroid hormone produced by the thyroid gland are thyroxine (T4) and triiodothyronine (T3), which are partially composed of iodine. The major form of thyroid hormone in the blood is thyroxine, which contain four atoms of iodine. To exert its effects, T4 is chemically converted to T3 by the removal of an iodine atom. Both T4 and T3 are used to treat thyroid hormone deficiency (hypothyroidism) and are regulated by TSH made by the thyrotropic cell of the anterior pituitary gland. T3 bound to carrier protein (eg, thyroxine binding glubuling: TBG, prealbumin, and albumin) and only small fraction circulates unbound or free T3.[1] The pituitary hormone TSH stimulates the thyroid gland to make and release the thyroid hormones (T4 and T3). When thyroid hormone levels decrease, the TSH rises and vice versa. In some cases of abnormal TSH values, measurement of T4 or T3 is performed to determine the extent of the thyroid abnormality.
An elevated T4 or T3, in association with a low or suppressed TSH, establishes hyperthyroidism.

An elevated TSH in conjunction with a low T4, is encountered in hypothyroidism. The T3 test is performed as part of an evaluation of thyroid function, combined with an interpretation of FT4 levels. T3 tests are often useful for diagnosis, monitoring hyperthyroidism and to determine the severity of the hyperthyroidism. Patients who are hyperthyroid will have the TSH level lower than normal and an elevated T3/T4 levels than normal. In some individuals with a low TSH, only the T3 is elevated and the free T4 (FT4) is normal. Accordingly, T3 testing is helpful in the hyperthyroid patient, since it is the last test to become abnormal.[2,3] The FREND™ Total T3 assay is to be used as an aid in the assessment of thyroid states, diagnosis of thyroid related disease.

Principle of the assay

A 70 µL specimen is added to pretreatment tube containing gold-T3 antibodies and incubated in the FREND™ AP System automatically, while triiodothyronine react with gold particles bound to antibodies against released triiodothyronine from carrier protein. After five minutes, the AP device transfers a 35 µL of sample to FREND™ Total T3 cartridge and incubated in AP system automatically. The cartridge is then placed into the FREND™ System, which is programmed to begin analysis once the sample has reacted with the reagents. The reaction and analysis time is ample, so a lower ratio of fluorescence correlates with a higher Total T3 concentration.

The FREND™ System is a bench-top fluorescence reader containing a touchscreen user interface. The System has a slot that accepts the FREND™ Total T3 test cartridge (which contains the reagents and sample), and is programmed to analyze the test when the sample has fully reacted with the on-board cartridge reagents. Results of the test are displayed on the screen and can be printed on an optional printer.

Material provided

Q'ty	Contents	Catalogue number
20	FREND™ Total T3 cartridge(s)	FRTT3AP 020
20	FREND™ Total T3 pretreatment tube(s)	
30	Disposable pipette tips	
01	FREND™ Total T3 Code chip	
01	FREND™ Total T3 Package Insert	

Materials required but not provided

- The FREND™ System
- The FREND™ AP
- Micro-pipetted capable of delivering 35 and 70 µL
- Personal protective equipment and biohazard waste equipment

Warning and Precautions

⚠ ***Caution:*** *Federal law restricts this device to sale by or on the order of a physician.*

- The FREND™ Total T3 cartridges are intended for *in vitro* diagnostic use only.
- The FREND™ Total T3 cartridges are only to be used on the FREND™ System.
- The FREND™ Total T3 cartridges are disposable, single use devices. Do not reuse them under any circumstances.
- Allow sealed cartridges to come to room temperature for 15~30 minutes prior to use.
- Cartridges and pretreatment tube should not be frozen.
- Assure the humidity in the laboratory is in the 10~80% range when tests are run.
- Assure the room temperature remains in the range of 22~30 °C when tests are run.
- Avoid cross-contamination between samples by using a new pipette tip for each new specimen.
- Avoid high humidity, direct sunlight or heat when storing cartridges and pretreatment tubes.
- Inaccurate results are possible if the sample used is contaminated in any way.

- Using specimens containing clotted fibrin could result in erroneous results.
- Over or under loading the cartridge with sample may result in inaccurate results.
- Human specimens are not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the lab may be derived from human materials.
  Please use Universal Precautions when handling all specimens and controls.
- Do not use the cartridges and pretreatment tubes beyond the expiration date on the pouch.
- Do not use the cartridge and pretreatment tubes if the pouch is damaged or the seal is broken.
- Perform testing as specified in the Package insert and User manual.
- Keep the cartridge and pretreatment tube sealed in the pouch until just ready for use.
- Use the cartridge and pretreatment tube immediately after opening the pouch.
- Wear disposable gloves when handling the cartridges, pretreatment tubes and the samples.
- Wash hands thoroughly and often after handling reagent cartridges or samples.
- Do not ingest the silica gel package in the cartridge pouch.
- The FREND™ Total T3 has been designed so that the high dose “hook effect” does not affect the vast majority of samples.
- Handle specimens in accordance with the OSHA Standard on Bloodborne Pathogens.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature. Reagent stability has been demonstrated for twelve months from the date of manufacture.
The expiration date is clearly indicated on the product box and the cartridges.

Materials	**Catalogue number**
Refrigerator temperature storage (2~8 ℃)	
FREND™ Total T3 cartridges	FRTT3AP 020
FREND™ Total T3 pretreatment tubes	None

Specimen collection and handling

Serum or lithium heparinized plasma is required for the assay. No special patient preparation is necessary. Collect the appropriate venous blood sample aseptically. For serum, allow the sample to clot for 30 minutes at room temperature. For lithium heparin, centrifuge after collection. Centrifuge the sample for 10 minutes at 3,000 rpm within 2 hours of collection and immediately separate the serum or plasma from the packed cells.

Separated Samples may be stored at 2-8 °C for up to 1 week prior to analysis. If the analysis is scheduled to be done more than 1 week after collection, the sample should be stored frozen at -20 °C or below for future use.

Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter such as fibrin clots or visible strands should be re-centrifuged before being tested. Prior to assay, slowly bring frozen samples to room temperature and mix gently but thoroughly before testing.

The sample required for the incubation step is 70 µL. The sample required for running the test on the FREND™ Total T3 cartridge is 35 µL.

For optimal results, avoid grossly hemolytic, lipemic, or turbid specimens. Specimens should be free of aggregated fibrin, red blood cells, or other particulate matter. When pipetting into the FREND™ Total T3 cartridge sample inlet, ensure that bubbles in the sample are avoided. Bubbles may restrict flow and result in an incomplete or erroneous test result.

Procedure

Calibration

There is no need for calibration to be performed by the end user.
All calibration statistics and information have been electronically stored on the FREND™ Total T3 Code chip included in each box of FREND™ Total T3 cartridges. The FREND™ Total T3 Code chip is specific for each manufactured lot of FREND™ Total T3 cartridges.

Always run external quality control samples to verify that the Total T3 results obtained on the FREND™ System meet the laboratory criteria for acceptability for each lot of FREND™ Total T3 cartridges.

Code chip installation

Please refer to the FREND™ System user manual for more detailed instructions relative to the Code chip installation. Abbreviated instructions follow here:

1) Insert the FREND™ System electrical cord into an appropriate outlet.
2) Insert the Code chip into the Code chip slot at the rear of the FREND™ System following the arrows.
3) Press the ‘Setup’ button on the ‘Main’ screen.
4) Press the ‘Code chip’ button on the ‘Setup’ screen.
5) The information embedded on the FREND™ Total T3 Code chip is automatically saved on the FREND™ System.
6) When the Code chip installation is completed, press the ‘OK’ button to go to the ‘Setup’ screen.
7) Press the ‘Item’ button on the ‘Setup’ screen.
8) Check the FREND™ Total T3 cartridge lot number and the installation date of the Code chip.
9) Press the ‘Home’ button to go to the ‘Main’ screen to begin running external quality control and patient samples.

Quality control

• FREND™ System QC Cartridge

FREND™ QC Cartridge contains multiple controls to check optic part of the system. By testing QC Cartridge, part of analytical components of the system of (1) laser power, (2) alignment, and (3) mechanical integrity are confirmed. For each day of patient testing perform QC Cartridge testing. Refer to the quality control procedures section in the User Manual of FREND™ System. In brief, perform QC Cartridge testing for the following conditions:

(1) Upon initial setup of the system,
(2) Each day of patient testing,
(3) When the system has been transported or moved,
(4) Whenever there is uncertainty about the performance of the system,
(5) Whenever required by your laboratory's quality control requirements.

• Internal procedural controls

The FREND™ Total T3 test cartridge contains built-in control feature. Fluorescence signal in the reference zone of each cartridge shows: (1) that enough volume is added, (2) that proper flow is obtained, and (3) that the antibody is reactive. If this reference zone signal is missing or lower than threshold, the FREND™ System consider it as an incorrect or failed test, not producing a test result but an error message. In addition, with each cartridge run, the system monitors, in part, for (1) flow of sample, (2) speed of sample flow, (3) shelf-life of cartridge components, (4) function of internal barcode scanner, and (5) function of scanner's mechanical components.

• External quality control testing

Commercially available controls from a variety of manufacturers are available that contain Total T3 as a measured analyte. It is recommended that a minimum of two (2) levels of controls be run at least once per month or once for each new lot, whichever comes earlier. However, Controls should be run with a minimum frequency, depending on number of tests run in the laboratory. Each laboratory should establish its own criteria based on the following parameters:

(1) Each new lot,
(2) Each new shipment (even if from the same lot previously received),
(3) Each new operator (an individual who has not run the tests for at least two weeks),

(4) Monthly, as a continued check on storage conditions,
(5) Whenever problems (storage, operator, or other) are identified,
(6) Or other times as required by your laboratory's standard QC procedures.

Individual laboratory policy will dictate exactly which control materials and lot numbers should be run, the frequency with which controls are to be tested, criteria for acceptance of the results and required corrective action to be taken if results do not meet laboratory criteria. If any external quality control sample values are out of the acceptable range, it will be necessary to investigate the problem before reporting patient results to assure there is not an instrument or software malfunction. Do not assay patient samples on the FREND™ System using FREND™ Total T3 if quality control on how to determine acceptability of external control material results. Each laboratory operates under a different set of regulations. Every laboratory must follow the standardized procedures acceptable to the regulatory agencies to which the laboratory is responsible.

Specimen processing

- **Preparation**

Remove sufficient cartridges and pretreatment tubes of FREND™ Total T3 from the refrigerator to test the number of patient samples and required external quality materials. Allow the tubes and the sealed pouches containing the cartridges to come to room temperature for 15~30 minutes prior to the start of the testing sequence. Heating block provided with FREND™ System should be turned on 7~8 minutes before use.

If using refrigerated patient samples, remove those from the refrigerator and allow to them to come to room temperature prior to testing. If frozen samples will be utilized, be sure these are removed from the freezer, thawed naturally and then mixed gently but thoroughly prior to testing. Testing should not begin on these previously frozen samples until they have reached room temperature.

There are no other reagents or sample preparations necessary.

• **Assay procedure**

Note: When processing samples, rinse the pipette several times with the sample and dispense 70 µL into the pretreatment tube.

1) Prepare the FREND™ Total T3 cartridges, pretreatment tube and specimen at room temperature (18~25 °C). Open the pouch and place FREND™ Total T3 cartridge into the cartridge tray of the AP device. Press **"NEXT"** to close the cartridge tray and open the pretreatment tube tray.
2) Transfer a 70 µL of specimen to the pretreatment tube.

⚠ ***Caution:*** *Once the sample is added to the pretreatment tube, do not invert the tube.*

Insert the pretreatment tube into the tube hole in the FREND™ AP pretreatment tube tray. Refer to the FREND™ AP User manual for complete operating instructions.

3) Press the **"NEXT"** button. The pretreatment tray will close and the first incubation step (5 minutes) will begin.
4) After the first incubation is complete, 35 µL of mixed sample will be loaded onto the cartridge and the second incubation step (2 minutes) will begin.
5) When both incubation steps are completed, the cartridge tray will open and the cartridge will be ready to be inserted into the FREND™ System.
6) Press the **'Test'** button on the **'Main'** screen of the FREND™ System.
7) The system moves to the Patient ID screen automatically.
8) Type the Patient ID and press the **'Enter'** button to begin the test.
9) Insert the cartridge into the cartridge slot using the cartridge arrow as a guide.

⚠ ***Caution:*** *Check the direction of the cartridge before insertion and assure the insertion is complete.*

10) When the reaction in the cartridge is completed, the FREND™ System will automatically begin the reading process.
11) When the measurements are completed, the cartridge will automatically be expelled and the results displayed.

⚠ ***Caution:*** *Do not disconnect power cord or shut off power on the FREND™ System while a cartridge is in the reading chamber. This may cause a system error.*

12) If the FREND™ System is connected to the optional printer, press the **'Print'** button and the results will be output on the printer paper. For more detailed instructions, please refer to the FREND™ System User Manual.

Procedural notes

Samples cannot be diluted for Total T3 determinations. Samples which read ">6.00 ng/mL" should be reported as such.

Calculation of results

The FREND™ System performs all sample and reagent handling operations automatically within the cartridge once the sample has been manually loaded to the sample inlet in the cartridge and the cartridge placed into the FREND™ System. The rate of fluorescence produced by the reaction is read at various intervals during the analysis process, blank reading are subtracted after which the net rate is automatically converted to Total T3 concentration in ng/mL based upon information stored on the FREND™ Total T3 Code chip.
This result is then output on the screen and to the optional printer.
It is also stored in memory on the FREND™ System.

Screen displayed for various concentration scenarios

Displayed result	Description
	Total T3 concentration Less than 0.40 ng/mL
	Total T3 concentration Not less than 0.40 ng/mL and not higher than 6.00 ng/mL
	Total T3 concentration Higher than 6.00 ng/mL

Limitations of the procedure

1) When used for diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g, symptoms, results of other tests, clinical impressions, medical history, therapy, etc.)
2) The FREND™ System paired with a FREND™ Total T3 cartridge, is programmed to report 6.00 ng/mL as the highest concentration of Total T3 measurable without dilution. The lowest measurable concentration is 0.40 ng/mL – the assay limit of quantitation.
3) Specimens from patients with heterophilic antibodies, such as anti-mouse (HAMA), anti-goat (HAGA), or anti-rabbit (HARA) antibodies, maybe show falsely elevated or depressed values or may result in an incomplete test.[11, 12] Patients routinely exposed to animals or animal serum products can be prone to these types of heterophilic interferences.
4) Certain medications may interfere with assay performance. All results should be interpreted with respect to the clinical picture of the patient.
5) Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.
6) Lipemia has an insignificant effect on the assay except in the case of gross lipemia where interference with the lateral flow of the sample in the cartridge may occur.
7) The concentration of Total T3 in a given sample determined with assays from different manufacturers can vary due to differences in assay methods, calibration, and antibody specificity.
8) Please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this package insert.
9) The FREND™ Total T3 has not been validated in point-of-care settings.
10) The FREND™ Total T3 is to be used in licensed clinical laboratories trained technicians.

Performance evaluation

Performance characteristics were evaluated for the FREND™ Total T3 as follows:

• Precision

A precision study for FREND™ Total T3 was performed as outlined in CLSI guideline EP5-A3. Four samples were assayed in two replicates, twice per day over a period of 20 days.

Sample ID	Concentration (ng/mL)	Within - run		Between - run		Between - day		Within - laboratory	
		SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
A	0.600	0.045	7.5	0.028	4.7	0.011	1.8	0.054	9.1
B	1.618	0.120	7.4	0.052	3.2	0.038	2.4	0.136	8.4
C	3.014	0.146	4.9	0.040	1.3	0.021	0.7	0.153	5.1
D	6.030	0.282	4.7	0.159	2.6	0.020	0.3	0.324	5.4

• Dilution linearity

The dilution linearity study as outlined in CLSI guideline EP6-A was performed by diluting a high concentration TT3 specimen. At each dilution level, the samples were tested in duplicate to determine the experimental value of TT3. Linearity was demonstrated from >0.40 ng/mL to <6.00 ng/mL. The measuring range for the FREND™ Total T3 is 0.40 ~ 6.00 ng/mL.

• Analytical sensitivity

The limit of blank (LoB) and limit of detection (LoD) were determined according to the CLSI guideline EP17-A2. LoB was determined by 60 replicates of a negative standard solution. LoD was determined by 12 replicates of five low level patient samples.

LoB (Limit of Blank)	LoD (Limit of Detection)	LoQ (Limit of Quantitation)
0.18 ng/mL	0.25 ng/mL	0.25 ng/mL

• Method comparison

The FREND™ Total T3 was compared to the predicate device using guidelines outlines in CLSI guideline EP9-A3. Samples (n=120) were measured in duplicate on both systems. Linear regression analysis demonstrated a slope of 0.9992 and correlation coefficient (R) of >0.99.

• Specificity

The following substances were evaluated for potential cross-reactivity with the FREND™ Total T3 at three concentrations. Testing was done according to the CLSI guideline EP7-A2. No significant cross-reactivity was found.

Substance	Concentration	Cross reactivity (%)		
		Low	Median	High
Thyroxine (T4)	100 ng/mL	107.7	99.0	94.3

• Interference

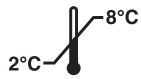
The interference study was performed as recommended in the CLSI guideline EP7-A2 using three concentrations of TT3. Recovery within 90% to 110% of the expected TT3 was considered as lack of interference. No interference by the substances below was found.

Substance	Concentration
Hemoglobin	500 mg/dL
Bilirubin	20 mg/dL
Triglyceride	2,000 mg/dL
Total protein	12 g/dL

References

1) Robbins J., Rall J.E. The Iodine-containing Hormones. In: Hormones in Blood (3rd Ed.). London: Academic Press, 1979,1:632-667.
2) Sterling K., Lazarus J.H. The thyroid and its control. *Annual Review of Physiology,* 1977, 39:349-371
3) Durr e Sabih, Mohammad Inayatullah. Managing thyroid dysfunction in selected special situations. *Thyroid Research,* 2013, 6:1-7

Glossary of symbols

	Do not reuse
	Use by YYYY-MM-DD
	Lot number/Batch number
	Catalog number/Reference number
	Consult Instructions for Use
	Manufacturer
	Authorized representative in the Europe Community
	*In vitro* diagnostic medical device
	Temperature limitation
	Contains sufficient for <n> tests
	Do not use if package ins damaged.
	For prescription use only
	CE marking
	Irritation

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