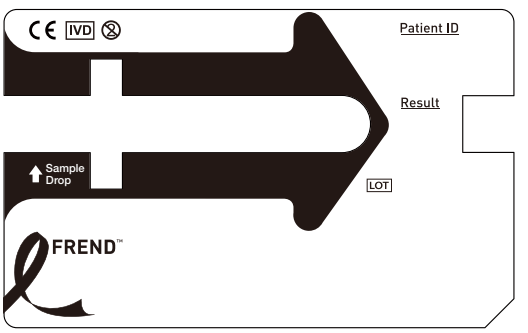
NanoEntek

NESPI-FRFT3-001EN (V.0.0)

CE IVD

FREND Free T3

REF FRFT3 020



EN

NanoEntek

NESPI-FRFT3-001EN(V.0.0)

IVD

FREND Free T3

Quantitative Assay for Free Triiodothyronine

REF FRFT3 020

IVD For *in vitro* diagnostic use only

1. Intended purpose

FREND Free T3 Test System is a rapid indirect competitive fluorescence immunoassay for the quantitative determination of Free Triiodothyronine (FT3) in human serum and lithium heparinized plasma specimens using the FREND™ System. Measurements of FT3 are used in the diagnosis of thyroid disorders. FREND Free T3 Test is intended for use in clinical laboratories. For in vitro diagnostic use only. For professional use only.

2. Summary and explanation of test

3,5,3' Triiodothyronine (T3) is a thyroid hormone with a molecular weight of approximately 651 daltons and a serum half-life of about 1.5 days. T3 circulates in the bloodstream as a dynamic equilibrium between protein-bound and free (unbound) forms. The majority of circulating T3 is bound to carrier proteins such as thyroxine-binding globulin (TBG), transthyretin (prealbumin), and albumin. Reported distribution among these proteins varies, with TBG accounting for roughly 38–80%, prealbumin 9–27%, and albumin 11–35% of T3 binding. Only a small fraction (approximately 0.2–0.4%) of total T3 exists in the unbound state, known as free T3 (FT3), which represents the biologically active component of the hormone.[1,2,3]

Free T3 plays a crucial role in regulating metabolism, growth, and development, and is especially important in conditions where thyroid

hormone binding capacity is altered. Measurement of FT3 provides clinical value in evaluating thyroid function, particularly in distinguishing various forms of hyperthyroidism. In Graves' disease, FT3 is often elevated more significantly than free thyroxine (FT4). In approximately 5% of hyperthyroid patients, only FT3 is elevated, a condition known as T3 thyrotoxicosis. Conversely, FT4 levels may be more prominently increased in toxic multinodular goiter or in cases of excessive T4 administration.[4,5,]

Serum FT3 measurements are valuable in the differential diagnosis of hyperthyroid states and may assist in monitoring the effectiveness of antithyroid therapy, which often aims to reduce both T3 production and peripheral conversion of T4 to T3. Additionally, FT3 levels can reflect the clinical severity of thyrotoxicosis and may aid in assessing thyroid function in patients where alterations in thyroid hormone-binding proteins complicate interpretation of total hormone measurements.[6,7,8]

This assay is intended for use in the quantitative determination of Free T3 in human serum or plasma, serving as an aid in the clinical assessment of thyroid status.

3. Principle of the assay

FREND Free T3 assay is a competitive immunoassay utilizing Fluorescent nanoparticle in microfluidic flow to capture and quantify Free T3 in serum and plasma specimens.

A 70 µl of specimen is added to pretreatment tube containing gold-T3 antibodies and incubated in the FREND™ AP System automatically, for five minutes, while triiodothyronine react with gold particles bound to antibodies against released triiodothyronine from carrier protein.

After five minutes, 35 µL of sample in the tube is transferred to the sample inlet of a single use FREND Free T3 test cartridge and allowed to equilibrate for 2 minutes in the FREND™ AP automatically. The cartridge is then placed into the FREND™ System, which is programmed to begin analysis time is approximately 8 minutes. Free T3 quantification is based on the ratio of fluorescence detected by the FREND™ System at the FREND Free T3 Test and Reference zones. The magnitude of the fluorescent ratio is inversely proportional to the amount of Free T3 in the sample, so a lower ratio of fluorescence correlates with a higher Free T3 concentration.

FREND™ System is a bench-top fluorescence reader containing a touchscreen user interface. The System has a slot that accepts the FREND Free 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.

4. Materials provided

Q'ty	Contents	Catalogue number
20	FREND Free T3 Cartridge(s)	FRFT3 020
20	FREND Free T3 Pretreatment tube(s)	
30	Disposable pipette tip(s)	
01	FREND Free T3 Code chip	
01	FREND Free T3 Package insert	

One cartridge contains:

T2-BSA
Anti-T3 antibody
Fluorescent particles

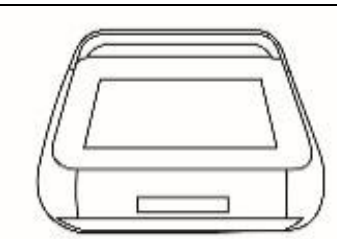
One pretreatment tube contains:

Anti-T3 antibody
Gold nano-particle

5. Materials required but not provided

The following materials are not provided with the reagent but are required to perform FREND Free T3 on the FREND™ system.

- FREND™ System

		
Model name	**F10**	**FREND 2.0**
Electrical rating	Voltage: 100–240V~ Current: 1.7A Frequency: 50/60 Hz	Voltage : 100–240V~ Current: 1.7A Frequency : 50/60 Hz

Electrical Input	Voltage: DC 12V Current: 3.33A	Voltage : DC 12V Current : 5.0A
Dimensions	240 mm X 260 mm X 175 (W X L X H) 9.4 inch x 10.2 inch x 6.9 inch	276 mm X 263 mm X 202 mm (W X L X H) 10.9 inch x 10.3 inch x 7.9 inch
Weight	3 kg (6.6 lbs)	3.4 kg (7.5 lb.)
Optical power	Class I	Class I
User interface	Active screen size: 7.0 inches diagonal dimension Pixel format: 800 x 480, TFT LCD	Active screen size: 8.0 inches diagonal dimension Pixel format: 1920 x 1080, Capacitive Touch
Operating condition	Temperature: 15℃–30℃ Humidity : 10 %–80 %	Temperature: 10℃–40℃ Humidity : 10 %–80 %
Storage condition	Temperature: 15℃–30℃ Humidity : 10%–80%	Temperature: 10℃–40℃ Humidity : 10%–80%

- FREND™ AP
- Micro-pipette, or any pipette, capable of delivering 70 µL
- Personal protective equipment and biohazard waste disposal containers

6. Warning and Precautions

1) General precautions

(1) For professional use only.

(2) The FREND Free T3 is disposable, single use devices. Do not reuse it under any circumstances.

(3) The FREND Free T3 is intended for in vitro diagnostic use only.

(4) Do not use the cartridge after the expiration date indicated on the pouch.

(5) The FREND Free T3 cartridges are only to be used on the NanoEntek FREND™ System.

(6) Assure the humidity in the laboratory is in the 10-80% range when tests are run.

(7) Avoid direct sunlight or heat in the testing area.

(8) Cartridgesand pretreatment tubes should not be frozen.

(9) Testing in a dusty environment may lead to erroneous test results. Maintain a testing area clean, free from dust and other potential contaminants.

(10) Perform testing as specified in the package insert and the user manual.

(11) Report any serious incidents that occur during or as a result of using the FREND Free T3 to the manufacturer and/or its authorized representative and to your national competent authority.

2) Precautions when testing

(1) Do not reuse.
(2) To differentiate a used cartridge, check the back side. The presence of the liquid sample in the cartridge channel indicates it has been used.
(3) Allow sealed cartridges and pretreatment tubes to come to the room temperature (20–30°C) for 15–30 minutes prior to use.
(4) Keep the cartridge sealed in the pouch until ready to use.
(5) Use the cartridge immediately after opening the pouch. However, if immediate use is not possible, it is recommended to use within 4 hours after opening.
(6) Do not use the cartridge if the pouch is damaged or the seal is broken.
(7) Avoid cross-contamination between samples by using a new pipette tip for each new specimen.
(8) Inadequate pipetting or inappropriate use may occur insufficient or excessive volume of the specimen into the cartridge which may affect test results.
(9) Always use a calibrated pipette. Using an uncalibrated pipette may cause inaccurate results.
(10) Do not ingest the silica gel packet found in the cartridge pouch.
(11) Do not bend cartridges.
(12) To ensure the correct cartridge is matched with the correct sample, write the Patient ID in the designated ‘Patient ID’ area on the cartridge.
(13) Do not write or scribble on the cartridge's barcode area.
(14) The reaction starts automatically once the sample is loaded into the cartridge inlet.
(15) The cartridge inserted wrong direction, the result can’t be appeared.

(16) The barcode on the cartridge damaged, FREND™ System show error code.

(17) For accurate results, insert the cartridge into the FREND™ System as soon as possible after loading the sample. The FREND™ System will automatically read the result when the reaction is complete. If more than 30 minutes have passed since you loaded the sample, discard the cartridge and re-test a new one.

3) Precautions for sample handling and storage

(1) Use Universal Precautions when handling all specimens and controls.
(2) Wear disposable gloves when handling the cartridges and the samples.
(3) Inaccurate results are possible if the sample used is contaminated in any way.
(4) Inadequate or inappropriate sample collection, storage, and transport may bring false test results.
(5) Wash hands thoroughly and often after handling reagent cartridges or samples.
(6) Handle the sample with caution as it may contain unknown viruses or bacterial pathogens that pose a risk.
(7) Samples stored in a refrigerated or frozen state should be brought to room temperature and thoroughly mixed before testing.

4) Precautions for result interpretation

The results should not be used as a sole basis for diagnosis. When interpreting the results, always consider them alongside the patient's medical history, clinical examination, and other relevant information.

5) Other precautions

The waste materials used in the examination should be disposed of by either subjecting them to high-pressure steam sterilization at 121℃ for at least 30 minutes or following the waste disposal standards

7. 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 12 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 Free T3 Cartridges	FRFT3 020
FREND Free T3 Pretreatment tubes	None
Room temperature storage (20–30 ℃)	
Pipette tips	None

8. Specimen collection and handling

Serum or lithium heparinized plasma is required for the assay. Other anticoagulants have not been validated for use with the FREND Free T3 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 Free 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 Free 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.

9. Procedure

1) 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 Free T3 Code chip included in each box of FREND Free T3 cartridges. The FREND Free T3 Code chip is specific for each manufactured lot of FREND Free T3 cartridges.

Always run external quality control samples to verify that the FT3 results obtained on the FREND™ System meet the laboratory criteria for acceptability for each lot of FREND Free T3 cartridges.

2) Code chip / QC code chip installation

There are two models available for the FREND™ System: F10 and FREND 2.0. Please refer to the specific model's user manual for more detailed instructions on Code chip installation. Abbreviated instructions are as follows:

- **FREND™ System (Model: F10)**

  ① Insert the FREND™ System (F10) electrical cord into an appropriate outlet.

  ② Press the '**Setup**' button on the '**Main**' screen.

  ③ Insert the Code chip into the Code chip slot at the rear of the system, following the arrow.

  ④ Press the '**Code chip**' button on the '**Setup**' screen.

  ⑤ The information embedded in the FREND Free T3 Code chip is automatically saved in the FREND™ System.

  ⑥ When the Code chip installation is complete, press the '**OK**' button to return to the '**Setup**' screen.

  ⑦ Press the '**Item**' button on the '**Setup**' screen.

  ⑧ Click the FREND Free T3 cartridge and check the installed lot number and the installation date of the Code chip.

  ⑨ Press the '**Home**' button to go to the '**Main**' screen to begin running the external quality control and the specimens.

- **FREND™ System (Model: FREND 2.0)**

  ① Insert the FREND™ System (FREND 2.0) electrical cord into an appropriate outlet.

  ② Press the '**Setting**' button on the '**Main**' screen.

  ③ Press the '**Code**' button on the '**Setting**' screen.

  ④ When installing with the Code chip, insert the Code chip into the Code chip slot on the right side of the system following the arrow. Then, press the '**Code chip**' button.

  ⑤ When installing with the QR code, press the '**QR Code**' button, then scan the QR code with the barcode scanner on the front of the system.

  ⑥ The information embedded in the FREND Free T3 Code chip is automatically saved in the FREND™ System.

  ⑦ When the Code chip installation is complete, press the '**X**' button to close the window.

  ⑧ Press the '**Item**' button on the '**Setting**' screen.

  ⑨ Click the FREND Free T3 cartridge and check the installed lot number and the installation date of the Code chip.

  ⑩ Press the '←**Back**' button to exit '**Setting**' menu and begin running the external quality control and the specimens.

3) Quality control

(1) FREND™ System QC Cartridges

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 specific model's User Manual of FREND™ System. In brief, perform QC cartridge testing for the following conditions

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

(2) Internal procedural controls

The FREND Free T3 test cartridge contains built-in control features. Fluorescence signal in the Control Zone of each cartridge shows: (1) that enough sample volume is added, (2) that proper flow is obtained, and (3) that the antibody is reactive. If this Control Zone signal is missing or lower than the threshold, the FREND™ System considers it as an incorrect or failed test, and produces an error message instead of a test result. 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.

(3) External quality control testing

Commercially available controls that contain FT3 as a measured analyte are available from a variety of manufacturers. 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 according to the local requirements for each laboratory. Each laboratory should establish its own criteria based on the following parameters.

- Each new lot,
- Each new shipment (even if from the same lot previously received),
- Each new operator (an individual who has not run the tests for at least two weeks),
- Monthly, as a continued check on storage conditions,
- Whenever problems (storage, operator, or other) are identified,
- 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 if 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 of software malfunction. Do not assay patient samples on the FREND™ System using FREND Free T3 if quality control results do not give expected values. Refer to your laboratory policies on how to determine acceptability of external control materials results. Every laboratory must follow the standardized procedures acceptable to the regulatory agencies to whom the laboratory is responsible.

4) Specimen processing

(1) Prepration

Remove sufficient FREND Free T3 cartridges and pretreatment tubes from the refrigerator to test the number of patient samples and required external quality control 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.

If using refrigerated patient samples, remove those from the refrigerator and allow 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 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 Free T3 cartridge, pretreatment tube, and specimen at room temperature (20–30℃). Open the pouch and place the FREND Free T3 cartridge into the cartridge tray of the AP device. Press **"NEXT"** to close the cartridge tray and open the pretreatment tube tray.
   To ensure the correct cartridge is matched with the correct sample, write the Patient ID in the designated 'Patient ID' area on the cartridge label.

    ***Caution: Do not write or scribble on the cartridge's barcode area.***
2) Transfer 70 µL of specimen to the pretreatment tube.

    ***Caution:*** *Once the sample is added to the pretreatment tube, do not invert the tube.*
3) 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.
4) Press the **"NEXT"** button. The pretreatment tray will close and the first incubation step (5 minutes) will begin.
5) After the first incubation is completed, 35µL of mixed sample will be

loaded onto the cartridge and the second incubation step (2 minute) will begin.

6) When both incubation steps are completed, the cartridge tray will open and the cartridge will be ready to be inserted into the FREND™ System.
7) Press the **'Test'** button on the **'Main'** screen of the FREND™ System.
8) The system moves to the Patient ID screen automatically.
9) Type the Patient ID and press the **'Enter'** button to begin the test.
10) 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.*

11) When the reaction in the cartridge is completed, the FREND™ System will automatically begin the reading process.
12) 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.*

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

10. Specimen Dilution Procedures

Samples cannot be diluted for FT3 determinations. Samples which read > 20.00 pg/mL should be reported as such.

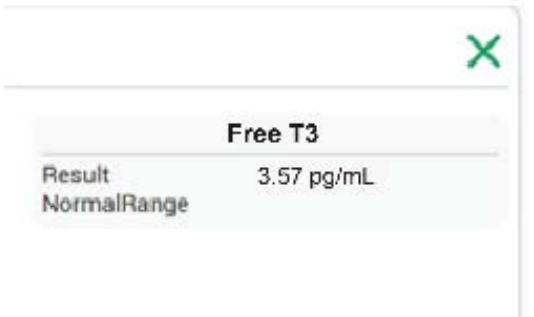
11. Calculation of results

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 readings are subtracted after which the net rate is automatically converted to FT3 concentration in ng/dL based upon information stored on the Free T3 Code chip. This result is then displayed on the screen and can be sent to the optional printer. It is also stored in memory on the FREND™ System.

Screen displayed for various concentration scenarios (F10)

Displayed result	Description
Date/Time : 2016-1-19 10:30 Patient ID: User ID : Order # : Lab ID: NANOENTEK Free T3 : < 1.50 pg/mL	Free T3 Concentration Less than 1.50 pg/mL
Date/Time : 2016-1-19 10:30 Patient ID: User ID : Order # : Lab ID: NANOENTEK Free T3 : 3.57 pg/mL	Free T3 Concentration Not less than 1.50 pg/mL And not higher than 20.00 pg/mL
Date/Time : 2016-1-19 10:30 Patient ID: User ID : Order # : Lab ID: NANOENTEK Free T3 : > 20.00 pg/mL	Free T3 Concentration Higher than 20.00 pg/mL

Screen displayed for various concentration scenarios (FREND 2.0)

Displayed result	Description
	Free T3 Concentration Less than 1.50 pg/mL
	Free T3 Concentration Not less than 1.50 pg/mL And not higher than 20.00 pg/mL
	Free T3 Concentration Higher than 20.00 pg/mL

12. 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 Free T3 cartridge, is programmed to report 20.00 pg/mL as the highest concentration of FT3 measurable without dilution. The lowest measurable concentration is 1.53 pg/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, may show falsely elevated or depressed values or may result in the error message **"Incomplete Test"**. Patients routinely exposed to animals or animal serum products can be prone to these types of heterophilic interferences. If the FT3 level is inconsistent with clinical evidence, additional FT3 or other thyroid testing using a different method is suggested to confirm the results.
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 FT3 in a given sample determined using 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 insert sheet.
9) FREND Free T3 has not been validated in point-of-care settings.
10) Performance of this assay has not been established with neonatal specimens or specimens from pregnant patients.
11) FREND Free T3 is to be used in licensed clinical laboratories with trained technologists.

13. Expected values

As with every clinical diagnostic test, a reference interval corresponding to the characteristics of the population being tested should be determined by each laboratory. Historically, it has been shown that there are no race- or gender-specific differences in the reference interval for FT3, so a single adult

reference interval is reasonable and justified. Serum samples from a total of 196 normal, apparently healthy adult individuals were assayed on 3 lots of the FREND Free T3 assay using a single FREND™ System. The reference interval was determined according to CLSI guideline C28-A3, was found to be 0.83–1.60 ng/dL. As in all in vitro diagnostic testing, a Free T3 result generated using the FREND Free T3 on the FREND™ System should be interpreted in the light other clinical findings and diagnostic procedures. Any FT3 results not correlating with the clinical condition should be repeated and other testing performed to clarify the situation

14. Performance characteristics

Ver.F10

Precision

A precision study for FREND Free T3 was performed as outlined in CLSI EP05-A3. Three samples were assayed in two replicates, twice per day over a period of 20 days.

Sample	Mean (pg/mL)	Repeatability (95% CIs)		Within-laboratory (95% Cis)	
		SD	CV (%)	SD	CV (%)
Cal. A	2.00	0.15 (0.12, 0.19)	7.0 (5.7, 9.0)	0.15 (0.13, 0.18)	7.4 (6.4, 8.8)
Cal. B	5.00	0.28 (0.23, 0.36)	5.4 (4.4, 6.9)	0.31 (0.27, 0.37)	5.9 (5.1, 7.1)
Cal. C	10.00	0.50 (0.41, 0.64)	5.1 (4.2, 6.6)	0.75 (0.64, 0.91)	7.7 (6.6, 9.3)

- Between-run (Reproducibility)

The results met with acceptance criteria in reproducibility test of FREND Free T3.

1) Between-Lot

Sample	Conc. (pg/mL)	Mean			Total Mean	SD	CV (%)
		Lot 1	Lot 2	Lot 3			
Cal. A	2.00	2.02	2.05	2.07	2.05	0.10	4.7
Cal. B	5.00	5.16	5.13	5.09	5.13	0.25	4.8
Cal. C	10.00	9.94	10.00	10.05	10.00	0.46	4.6

2) Between-Operator

Sample	Conc. (pg/mL)	Mean			Total Mean	SD	CV (%)
		Tester 1	Tester 2	Tester 3			
Cal. A	2.00	2.05	2.05	2.11	2.07	0.09	4.4
Cal. B	5.00	5.08	5.24	5.12	5.15	0.21	4.7
Cal. C	10.00	10.31	10.41	10.30	10.34	0.27	2.6

3) Between-Site

Sample	Conc. (pg/mL)	Mean			Total Mean	SD	CV (%)
		Site 1	Site 2	Site 3			
Cal. A	2.00	2.08	2.10	2.06	2.08	0.11	5.1
Cal. B	5.00	5.15	5.17	5.20	5.17	0.24	4.7
Cal. C	10.00	10.15	10.24	10.17	10.19	0.31	3.0

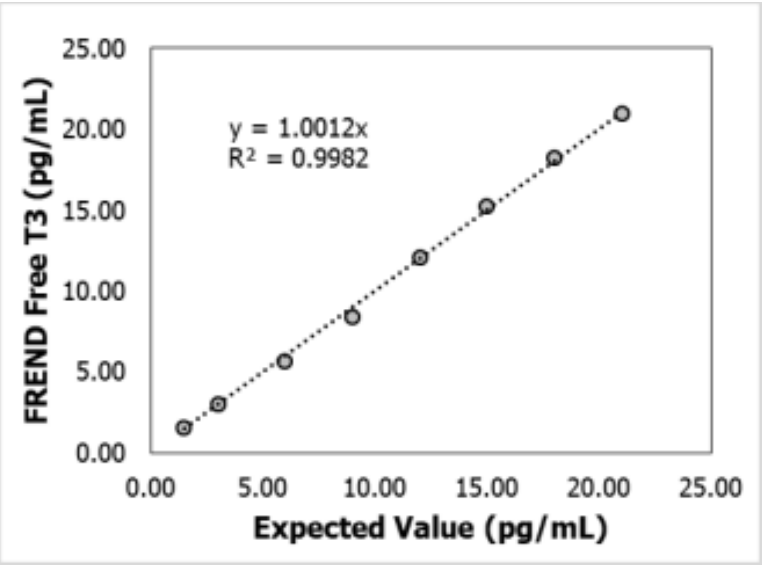
4) Between-Instrument

Sample	Conc. (pg/mL)	Mean			Total Mean	SD	CV (%)
		Device 1	Device 2	Device 3			
Cal. A	2.00	2.01	2.10	2.09	2.07	0.09	4.4
Cal. B	5.00	4.98	4.91	5.01	5.01	0.27	5.4
Cal. C	10.00	9.95	9.94	9.91	9.94	0.28	2.8

Linearity

The dilution linearity study as outlined in CLSI EP06-A2 was performed by dilution a high concentration FREND Free T3 specimen with Free T3 depleted serum. Linearity was demonstrated from 1.50 to 20.00 pg/mL.

FREND Free T3 – Linearity



The analytical measurement range of FREND Free T3 is from 1.50 pg/mL to 20.00 pg/mL.

Sensitivity

The limit of blank (LoB) and limit of detection (LoD) was determined using guidelines found in CLSI document EP17-A. LoB was determined from 60 replicate measurements using a negative standard solution. LoD was determined suing 12 replicates measurements of five low patient samples.

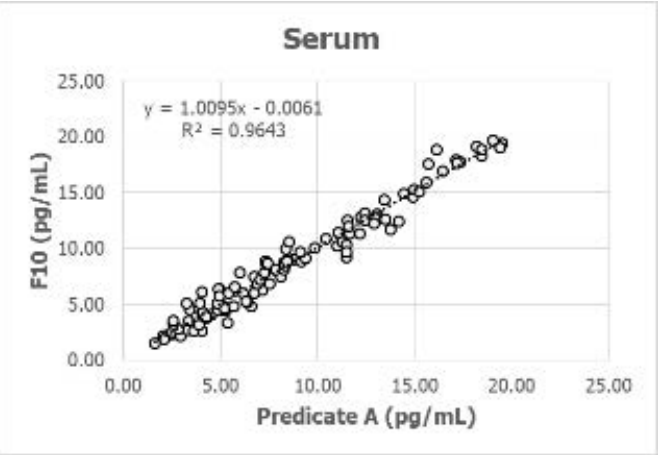
FREND	LoB	LoD	LoQ
F10	1.32 pg/mL	1.50 pg/mL	1.44 pg/mL

Method comparison

FREND Free T3 was compared to the predicate device using guidelines outlined in CLSI document EP09-A3. Samples (n=106) were measured in duplicate on both systems. Linear regression analysis demonstrated a correlation coefficient (R^2) of $\geq$ 0.98.

1) F10 vs Predicate device

Samples(N)	Slope	Intercept	Correlation coefficient (R)
106	1.0095	-0.0061	0.9820

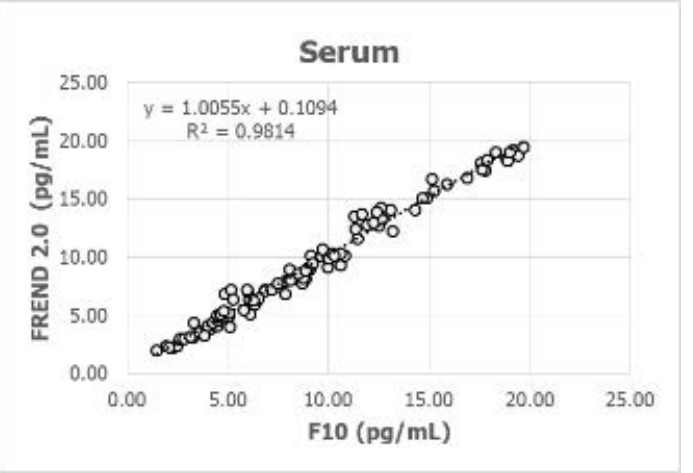


Method comparison

FREND Free T3 was evaluated with 'FREND 2.0' and predicate device 'F10' using CLSI document EP09-A3. According to the result of the study, FREND 2.0 has a high correlation with the predicate devices in FREND Free T3. The result of the study are as follows.

1) F10 vs FREND 2.0

Samples(N)	Slope	Intercept	Correlation coefficient (R)
106	1.0055	0.1094	0.9907



Ver.FREND 2.0

Precision

A precision study for FREND Free T3 was performed as outlined in CLSI EP05-A3. Three samples were assayed in two replicates, twice per day over a period of 20 days.

Sample	Mean (pg/mL)	Repeatability (95% CIs)		Within-laboratory (95% CIs)	
		SD	CV (%)	SD	CV (%)
Cal. A	2.00	0.11 (0.09, 0.15)	5.6 (4.6, 7.2)	0.12 (0.10, 0.14)	5.8 (5.0, 6.9)
Cal. B	5.00	0.28 (0.23, 0.35)	5.3 (4.3, 6.7)	0.31 (0.27, 0.37)	5.9 (7.0, 7.0)
Cal. C	10.00	0.28 (0.23, 0.36)	2.8 (2.3, 3.6)	0.30 (0.26, 0.35)	3.0 (2.6, 3.5)

- Between-run (Reproducibility)

The results met with acceptance criteria in reproducibility test of FREND Free T3.

1) Between-Lot

Sample	Conc. (pg/mL)	Mean			Total Mean	SD	CV (%)
		Lot 1	Lot 2	Lot 3			
Cal. A	2.00	2.04	2.00	2.02	2.02	0.09	4.6
Cal. B	5.00	5.28	5.34	5.29	5.30	0.28	5.2
Cal. C	10.00	10.00	10.06	9.85	9.97	0.28	2.8

2) Between-Operator

Sample	Conc. (pg/mL)	Mean			Total Mean	SD	CV (%)
		Tester 1	Tester 2	Tester 3			
Cal. A	2.00	1.97	2.02	2.04	2.01	0.10	5.0
Cal. B	5.00	5.30	5.22	5.27	5.26	0.30	5.7
Cal. C	10.00	10.05	10.02	10.06	10.04	0.28	2.8

3) Between-Site

Sample	Conc. (pg/mL)	Mean			Total Mean	SD	CV (%)
		Site 1	Site 2	Site 3			
Cal. A	2.00	2.03	1.99	1.94	2.01	0.09	4.3
Cal. B	5.00	5.19	5.27	5.29	5.25	0.25	4.7
Cal. C	10.00	10.06	10.18	9.88	10.04	0.30	2.9

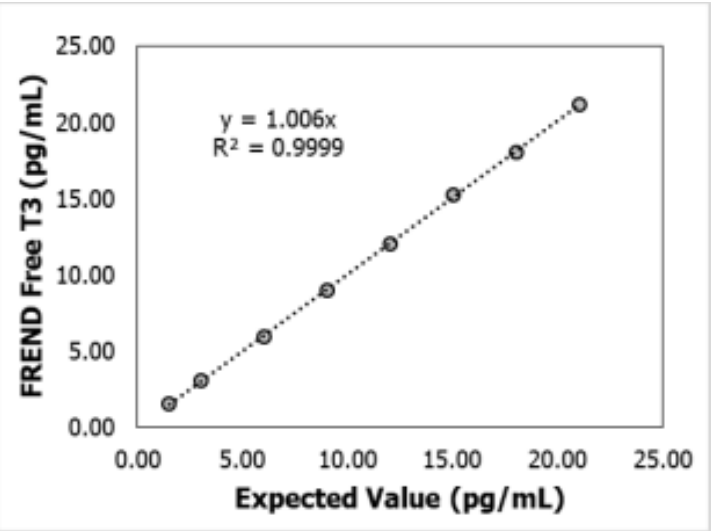
4) Between-Instrument

Sample	Conc. (pg/mL)	Mean			Total Mean	SD	CV (%)
		Device 1	Device 2	Device 3			
Cal. A	2.00	2.04	2.10	2.09	2.08	0.11	5.1
Cal. B	5.00	5.15	5.10	5.10	5.12	0.27	5.2
Cal. C	10.00	9.91	9.82	9.83	10.36	0.22	2.1

Linearity

The dilution linearity study as outlined in CLSI EP06-A2 was performed by dilution a high concentration FREND Free T3 specimen with Free T3 depleted serum. Linearity was demonstrated from 1.50 to 20.00 pg/mL.

FREND Free T3 – Linearity



The analytical measurement range of FREND Free T3 is from 1.50 pg/mL to 20.00 pg/mL.

Sensitivity

The limit of blank (LoB) and limit of detection (LoD) was determined using guidelines found in CLSI document EP17-A. LoB was determined from 60 replicate measurements using a negative standard solution. LoD was determined suing 12 replicates measurements of five low patient samples.

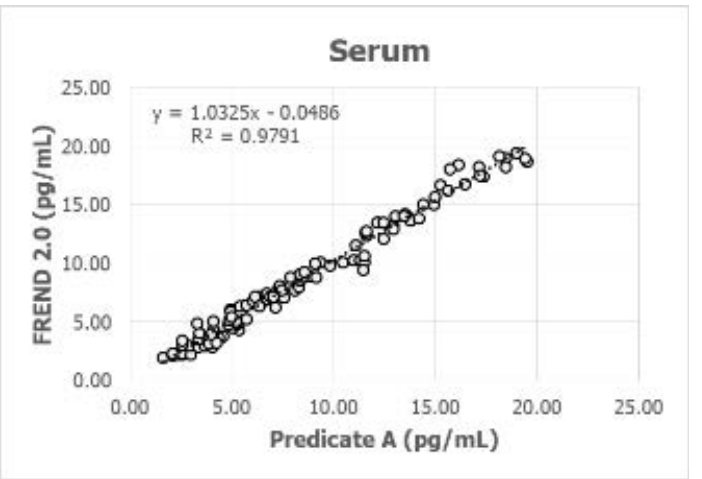
FREND	LoB	LoD	LoQ
FREND 2.0	1.25 pg/mL	1.35 pg/mL	1.37 pg/mL

Method comparison

FREND Free T3 was compared to the predicate device using guidelines outlined in CLSI document EP09-A3. Samples (n=106) were measured in duplicate on both systems. Linear regression analysis demonstrated a correlation coefficient (R^2) of $\geq$ 0.98.

1) FREND2.0 vs Predicate device

Samples(N)	Slope	Intercept	Correlation coefficient (R)
106	1.0325	-0.0486	0.9895



Method comparison

FREND Free T3 was evaluated with 'FREND 2.0' and predicate device 'F10' using CLSI document EP09-A3 According to the result of the study, FREND 2.0 has a high correlation with the predicate devices in FREND Free T3. The result of the study are as follows.

1) F10 vs FREND 2.0

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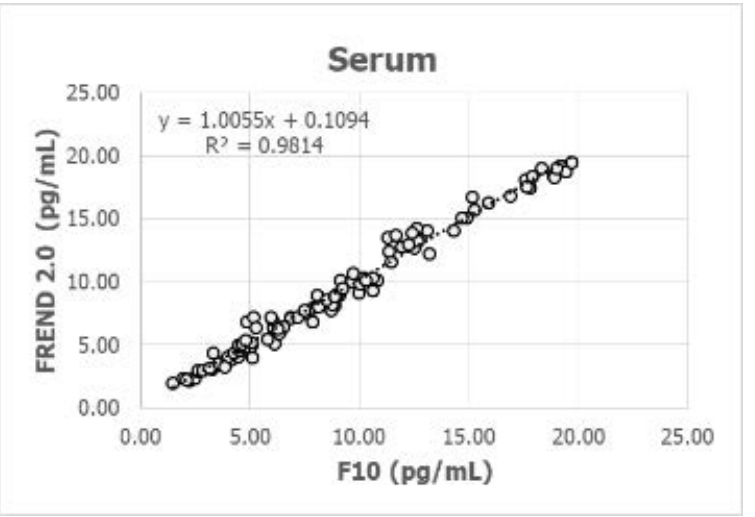

Ver.F10, FREND2.0

Interference

The interference study was performed as recommended in the CLSI EP07-A2 protocol using three concentrations of FT3. Recovery within 90% to 110% of the expected FT3 was considered as lack of interference. No interference by the substances below was found.

No.	Potential interfering	Concentration
1	Bilurubin, conjugated	20 mg/dL
2	Bilirubin, unconjugated	20 mg/dL
3	Biotin	0.12 mg/dL
4	Cholesterol	500 mg/dL
5	Hemoglobin	50 mg/dL
6	IgG	5 g/dL
7	IgM	1 g/dL
8	Intralipid	2,000 mg/dL

9	Rheumatoid factors	95 IU/mL
10	Triglycerides	3,000 mg/dL

Cross-reactivity

The following substances were evaluated for potential cross-reactivity with the FREND Free T3 at two concentrations. Testing was done according to the CLSI protocol EP07-A2. No significant cross-reactivity was found.

No.	Potential cross reactants	Concentration
1	3,3',5,5'-tetraiodothyroacetic acid	10 ng/dL
2	3-iodo-L-tyrosine	10 µg/mL
3	L-thyroxine	50 ng/mL
4	Reverse T3	1,000 pg/mL

Drug Interference

The interference study was performed as recommended in the CLSI EP07-A2, EP37 protocol using three concentrations of FT3. Recovery within 90% to 110% of the expected FT3 was considered as lack of interference. No interference by the substances below was found.

No.	Drug potential interfering	Concentration
1	Acetaminophen	1,324 µmol/L
2	Aspirin	300 µg/mL
3	Furosemide	181 µmol/L
4	Ibuprophen	2,425 µmol/L
5	Methimazole	2 µg/mL
6	Sodium salicylate	50 mg/dL
7	Propylthiouracil	10 µg/mL

Accuracy

The accuracy studies was evaluated on the FREND Free T3 assay following the CLSI guideline EP15-A2. As a result of the accuracy test for FREND Free T3, all samples met the criteria(±10%).

15. References

1) Cigdem, T.B., Merve, Y., Elif, K. Free triiodothyronine to free thyroxine ration in the differential diagnosis of thyrotoxicosis and hyperthyroidism: A tertospective study. International Journal of clinical practive, 2021, 75(5), e14003
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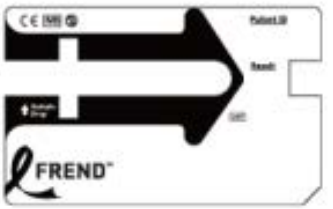
4) Spencer, C.A., & Wang, C.C. thyroglobulin measurement: techniques, clinical benefits, and pitfalls. Endocrinology and Metabolism clinics of North America, 1995, 24(4), 841–863.
5) C Ferrari, M Romussi, P Rampini, R Benco, M Boghen, A paracchi, S Frezzati, Serum free thyroid hormones in T3-toxicosis: a study of 35 patiens, 1983, 6(1), 55–58.
6) Yanchun L., Amy E.R., Ann M G., Christopher W.F., Limited utility of Free triiodothyronine testing, 2023, 8(5), 847–855.
7) Ross, D.S. Serum Thyroid-stimulating hormone measurement for assessment of thyroid function and disease, Endocrinology and Metabolism clinics of North America, 2001, 30(2), 245-264
8) Biondi, B., Cooper, D.S. The clinical significance of subclinical thyroid dysfunction. Endocrine Reviews, 2008, 29(1), 76–131.

Glossary of Symbols

	Caution, warning, Consult accompanying documents
REF	Catalogue number/Reference number
 www.nanoentek.com/eifu.php	Consult Instructions for Use An electronic instructions for use (eIFU) indicator (website address) may accompany the symbol when used to indicate an instruction to consult an eIFU.
LOT	Lot number/Batch number
	Use by YYYY-MM-DD or YYYY-MM
	Manufacturer
CE	CE marking
IVD	*In vitro* diagnostic medical device
	Temperature limitation
	Contains sufficient for <n> tests
	Do not reuse
	Do not use if package is damaged
Rx Only	For prescription use only CAUTION: Federal (U.S.) law restricts this device to sale by or on order of a physician.
US Corporation	US Corporation
Patient ID	Patient ID
Result	Result
Sample Drop	Sample Drop
EC REP	Authorized representative in the European Community

UK Representative	Authorized representative in United Kingdom
CH REP	Authorized representative in Switzerland
BRH	Authorized representative in Brazil

Kit Contents



Cartridge

Pretreatment tube

Pipette tip

Code chip

Package insert

Revised on 2025.06

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