VIDAS® FT4 (FT4N)

IVD

VIDAS® FT4 is an automated quantitative enzyme immunoassay for use on the instruments of the VIDAS® family, for the determination of free thyroxine (FT4) in human serum or plasma (lithium heparin), using the ELFA technique (Enzyme Linked Fluorescent Assay). Measurement of Free Thyroxin is intended for use as an aid in the diagnosis and treatment monitoring of thyroid disorders.

SUMMARY AND EXPLANATION

From the moment it is secreted into the blood stream, thyroxine or tetraiodothyronine (T4), produced by the thyroid gland, is predominantly (> 99.9%) bound to carrier proteins: TBG (Thyroxine Binding Globulin), TBPA (Thyroxine Binding PreAlbumin), albumin. The fraction that remains free (FT4) is considered as the active part of the hormone (1). The mechanisms regulating thyroid function have a direct effect on the concentration of this free fraction, which explains why it is relatively independent of the concentration of carrier proteins (2-3).

In patients with hyperthyroidism, the FT4 concentration increases, whereas in patients with hypothyroidism it generally decreases.

Patients on hormone replacement therapy (LT4) may have an elevation of FT4, although clinically they are euthyroid.

The VIDAS® FT4 test aids in diagnosing thyroid disorders.

The FT4 assay must be used in conjunction with other tests, such as TSH, as well as a clinical examination of the patient (4).

PRINCIPLE

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA).

The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR® several times.

The sample is collected and transferred into the well containing an alkaline phosphatase-labeled anti-T4 antibody (conjugate). The antigen present in the sample and the T4 antigen coated on the interior of the SPR® compete for the available sites on the specific anti-T4 antibody conjugated to alkaline phosphatase.

During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR®. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

CONTENT OF THE KIT (60 TESTS):

60 FT4N Strips	STR	Ready-to-use.
60 FT4N SPR®s 2 x 30	SPR®	Ready-to-use. Interior of SPR®s coated with thyroxine.
FT4N Control 1 x 2 mL (liquid)	C1	Ready-to-use. Human serum* + L-thyroxine + sodium azide (1 g/L). MLE data indicate the confidence interval in pmol/L ("Control C1 Dose Value Range").
FT4N Calibrator 1 x 2 mL (liquid)	S1	Ready-to-use. Human serum * + sodium azide (1 g/L). MLE data indicate the concentration in pmol/L ("Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value ("Calibrator (S1) RFV Range").
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or • MLE bar code printed on the box label.		
1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

* This product has been tested and shown to be negative for HBs antigen, antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

The SPR

The interior of the SPR® is coated during production with thyroxine. Each SPR® is identified by the FT4N code. Only remove the required number of SPR®s from the pouch and **carefully reseal the pouch after opening**.

The Strip

The strip consists of 10 wells covered with a labeled foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorimetric reading is performed. The wells in the center section contain the various reagents required for the assay.

Description of the FT4N strip

Wells	Reagents
1	Sample well.
2 - 3 - 4	Empty wells.
5	Conjugate: alkaline phosphatase-labeled anti-T4 antibody + 1 g/L Methylisothiazolone (MIT) (400 µL).
6	Wash buffer: Tris-NaCl (0.05 mol/L) pH 7.4 + 1 g/L Methylisothiazolone (MIT) (600 µL).
7	Wash buffer: Tris-Tween, NaCl (0.05 mol/L) pH 7.4 + 1 g/L Methylisothiazolone (MIT) (600 µL).
8	Wash buffer: diethanolamine* (1.1 mol/L or 11.5%) pH 9.8 + 1 g/L sodium azide (600 µL).
9	Empty well.
10	Reading cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + diethanolamine** (0.62 mol/L or 6.6%, pH 9.2) + 1 g/L sodium azide (300 µL).

* Signal Word: **DANGER**

Hazard statement

H318 : Causes serious eye damage.
H373 : May cause damage to organs through prolonged or repeated exposure.
H315 : Causes skin irritation.
H302 : Harmful if swallowed.

Precautionary statement

P280 :Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P309 + P311 : IF exposed or if you feel unwell: Call a POISON CENTER or doctor/physician.

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For further information, refer to the Material Safety Data Sheet.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Pipette with disposable tip to dispense 100 µL.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User's Manual.
- VIDAS family of instruments.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- **This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (see Laboratory Biosafety Manual - WHO - Geneva - latest Edition).**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- Do not use SPR®s if the pouch is pierced.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents (or disposables) from different lots.
- Use **powderless** gloves as powder has been reported to cause false results for certain enzyme immunoassay tests.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- The wash buffer in well 8 contains a harmful agent (11.5% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- The substrate in well 10 contains an irritant agent (6.6% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User's Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User's Manual).

STORAGE CONDITIONS

- Store the VIDAS® FT4 kit at 2-8°C.
- **Do not freeze reagents.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR® pouch is correctly sealed and undamaged. If not, do not use the SPR®s.
- **Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPR®s and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.

SPECIMENS

Specimen type and collection:

Human serum or plasma (lithium heparin).
Do not use EDTA tubes.

Types of tubes validated:

- Silicone coated glass tube,
- Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with lithium heparin,
- Plastic tube with lithium heparin and separation gel.

Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.
It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Specimen preparation

Plain tubes: wait for samples to coagulate and **centrifuge** according to the tube manufacturer's recommendations to eliminate fibrin.
Other tubes: follow the tube manufacturer's recommendations for use.
Frozen-stored samples: after thawing, all these samples must be homogenized before testing. Mix using a vortex-type mixer. Clarify the samples before by centrifugation, if necessary.

Specimen-related interferences

None of the following factors have been found to significantly influence this assay:

- hemolysis (after spiking samples with hemoglobin, from 0 to 300 µmol/L (monomer)),
- lipemia (after spiking samples with lipids, from 0 to 18 g/L equivalent in triglycerides),
- bilirubin (after spiking samples with bilirubin, from 0 to 480 µmol/L).

However, it is recommended not to use clearly hemolyzed, lipemic or icteric samples and, if possible, to collect a new sample.

Specimen stability:

Samples can be stored at 2-8°C in stoppered tubes for up to 8 days; if longer storage is required, freeze the sera or plasma at -25 ± 6°C. Serum-type samples can be stored for 6 months at -25 ± 6°C, with 4 freeze/thaw cycles.
Samples collected in lithium heparin must not be stored for more than 4 months at -25 ± 6°C, with 2 freeze/thaw cycles.

INSTRUCTION FOR USE

For complete instructions, see the User's Manual.
Reading VIDAS® Protocole Test Change (PTC) protocol data and MLE data

When using the assay for the first time:
With the external instrument barcode reader,
1. Scan the PTC barcode(s) at the end of the package insert. or downloadable from www.biomerieux.com/techlib. This reading allows VIDAS® PTC protocol data to be transferred to the instrument software for its update.
2. Scan the MLE data on the box label.

Note: If the MLE data have been read before the VIDAS® PTC protocol, read the MLE data again.

When opening a new lot of reagents:
Enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.
If this operation is not performed before initiating the tests, the instrument will not be able to print results.
Note: the master lot data need only be entered once for each lot.
It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User's Manual).

Calibration

Calibration, using the calibrator provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered. Calibration should then be performed every 14 days. This operation provides instrument-specific calibration information and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The calibrator, identified by "S1", must be tested **in duplicate** (see User's Manual). The calibrator value must be within the set RFV "Relative Fluorescence Value" range. If this is not the case, recalibrate.

Procedure

1. **Only remove the required reagents from the refrigerator. They can be used immediately.**
2. Use one "**FT4N**" strip and one "**FT4N**" SPR® from the kit for each sample, control or calibrator to be tested. **Make sure the storage pouch has been carefully resealed after the required SPR®s have been removed.**
3. The test is identified by the "**FT4N**" code on the instrument. The calibrator must be identified by "S1", and tested **in duplicate**. If the control is to be tested, it should be identified by "C1".
4. If necessary, clarify the samples by centrifugation.
5. Mix the calibrator, control and samples using a vortex-type mixer (for serum or plasma separated from the pellet).
6. **For this test, the calibrator, control, and sample test portion is 100 µL.**
7. Insert the "**FT4N**" SPR®s and "**FT4N**" strips into the instrument. Check to make sure the color labels with the assay code on the SPR®s and the Reagent Strips match.
8. Initiate the assay as directed in the User's Manual. All the assay steps are performed automatically by the instrument.
9. Reclose the vials and return them to 2–8°C after pipetting.
10. The assay will be completed **within approximately 40 minutes.** After the assay is completed, remove the SPR®s and strips from the instrument.
11. Dispose of the used SPR®s and strips into an appropriate recipient.

RESULTS AND INTERPRETATION

Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR® is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR®. The RFV (Relative Fluorescence Value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.
Results are automatically calculated by the instrument using calibration curves stored in memory (4-parameter logistic model) and are expressed in pmol/L.

Sera for free hormone assays should not be diluted. The result must be expressed as > 100 pmol/l.
VIDAS® FT4 assay results should be interpreted as part of a complete clinical evaluation and thyroid function assessment, including at least TSH determination.

QUALITY CONTROL

A control is included in each VIDAS® FT4 kit.
This control must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using this control. The instrument will only be able to check the control value if it is identified by C1.
Results cannot be validated if the control value deviates from the expected values.

Note

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

- Interference may be encountered with certain sera containing antibodies directed against the reagent components. For this reason, assay results should be interpreted taking into consideration the patient's history and the results of any other tests performed.
- Certain drugs may interfere with free thyroid hormone assays (5, 6, 7).

RANGE OF EXPECTED VALUES

As a guideline, 95% of the values corresponding to 623 adults who meet the selection criteria for establishing euthyroid status are within the range: 10.6 – 19.4 pmol/L.
It is recommended that each laboratory establishes its own reference values from a rigorously selected population (8).

PERFORMANCE

Studies performed using VIDAS® FT4 gave the following results:

Measurement range

The measurement range of the VIDAS® FT4 reagent is from 1 to 100 pmol/L.

Quantitation limits

The Limit of Quantitation (LoQ) is the lowest concentration of free T4 that can be quantified with a level of acceptable accuracy and precision.
LoQ = 1.11 pmol/L.
The study was performed as recommended by the CLSI® document EP17-A.

Precision

Seven samples were tested in duplicate in 40 different runs (2 runs per day) with 2 reagent lots at 3 sites (n=240).

Repeatability (within-run precision), and reproducibilities (within-system within-lot and between-system between-lot) were calculated using this protocol, based on the recommendations of the CLSI® document EP5-A2:

		Repeatability		**Within-system within-lot reproducibility**		**Between-system between-lot reproducibility**	
Sample	Mean concentration (pmol/L)	Standard deviation	CV (%)	Standard deviation	CV (%)	Standard deviation	CV (%)
Sample 1	**4.1**	0.25	6.2	0.42	10.3	0.54	13.2
Sample 2	**10.21**	0.37	3.6	0.67	6.6	0.84	8.3
Sample 3	**10.39**	0.39	3.8	0.54	5.2	1.35	13.0
Sample 4	**19.84**	0.61	3.1	0.86	4.3	1.46	7.4
Sample 5	**33.15**	0.82	2.5	1.18	3.6	1.68	5.1
Sample 6	**51.53**	1.18	2.3	1.96	3.8	3.02	5.9
Sample 7	**74.47**	2.25	3.0	2.98	4.0	4.86	6.5

Specificity

Interference was studied according to the recommendations of the CLSI® document EP7-A2.

Tested compound	No interference observed up to the concentration of:
3,5,-diiodothyrosine	199.25 µg/L
3,5-diiodothyronine	331.38 µg/L
L-Triiodothyronine	16.42 µg/L
D-thyroxine	2.54 µg/L

Comparison with another test method

587 serum samples distributed over the measurement range were assayed simultaneously using the VIDAS® FT4 kit and an enzyme immunoassay kit according to the recommendations of the CLSI® document EP9-A2.
The Passing and Bablok line equation obtained is:
Y = 1.067 X – 1.123
Correlation coefficient = 0.978 (n = 587)

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

1. SAPIN R., D'HERBOMEZ M. - Dosage des hormones thyroïdiennes : thyroxine (T4) et triiodothyronine (T3). In La thyroïde, LECLERE J., ORGIAZZI J., ROUSSET B., SCHLIENGER Elsevier, ed. 2001, 268-274.
2. PEARCE C.J., BYFIELD P.G.H. - Free thyroïd hormone assays and thyroïd function. Ann. Clin. Biochem., 1986, 23, 230-237.
3. EKINS R. - Measurement of Free Hormones in Blood - Endocrine Reviews, vol. 11, n°1, 1990, 5-46.
4. CARAYON P., NICCOLI-SIRE P., LEJEUNE P.J., et al. - Recommandation de consensus sur le diagnostic et la surveillance des maladies de la glande thyroïde. Ann. Biol. Clin.- mai-juin 2002, vol. 60, n°3.
5. STOCKIGT J. R. - Drug effects on thyroid function. Thyroid international 2 (2000).
6. GRONROOS P. E., IRJALA K. M., SELEN G. P., FORSSTROM J. J. – Computerized monitoring of potentially interfering medication in thyroid function diagnostics. Int J. Clin. Monit. Comput., 1997, 14(4), 255-259.
7. Norme CLSI. Measurement of Free Thyroid Hormones: Approved Guideline CA45-A V24 N°31.
8. Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease. Laboratory Medicine Practice Guidelines Editors : L. M. Demers, C. A. Spence, 2002, 1-32.

INDEX OF SYMBOLS

Symbol	Meaning
	Catalog number
	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories :

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user
Note:	*Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	9300800E	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (60 TESTS) WARNINGS AND PRECAUTIONS INSTRUCTIONS FOR USE

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