NanoEnTek

NESPI-FRTEAP-002EN(V.0.0)

CE IVD

FREND™ Testosterone

Total Testosterone

Intended use

The FREND™ Testosterone test system a fluorescence immunoassay designed for *in vitro* quantitative measurement of total testosterone in human serum and plasma (K_3 EDTA and lithium heparin). Measurements of testosterone are used in the diagnostic and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism impotence in males and, in females, hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries, and adrenogenital syndromes.

The FREND™ Testosterone microfluidic flow cartridge is designed for use in the FREND™ System fluorescent immunoassay reader. The FREND™ Testosterone test system is intended for use in clinical laboratories. For *in vitro* diagnostic use only. The test is not intended for use in point-of-care settings.

Summary and explanation of test

Testosterone is a male sex hormone secreted by the testes and regulated via its negative feedback on the secretion of luteinizing hormone by the pituitary gland. In females, testosterone is produced mainly by conversion of prohormones.

Circulating testosterone is 98% protein-bound in males, with slightly less being bound in females. The proteins responsible for biding testosterone are serum albumin and Sex Hormone Binding Globulin (SHBG), also referred to as Testosterone Binding Globulin (TeBG).[1]

Clinically, testosterone monitoring is used to help diagnose and differentiate endocrine disorders. In males, these include hypogonadism, testicular failure, infertility, hypopituitarism and hyperprolactinemia. In females, changes in serum testosterone levels can be caused by polycystic ovary syndrome, adrenal hyperplasia, infertility, hirsutism, amenorrhea, obesity and virilization.

Principle of the Assay

The FREND™ System includes a bench-top fluorescence reader with a slot that accepts the FREND™ Testosterone test cartridge. The instrument includes a touchscreen interface and is programmed to interpret 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.

To perform the test, add 70 µL (2 times in 35 µL) samples to the pretreatment tube using pipette and insert the Testosterone cartridge and pretreatment tube to FREND™ AP (Advanced Preparing device) according to the instructions. The sample is automatically mixed in the pretreatment tube and mixed sample is automatically loaded into the cartridge. The incubated cartridge in the FREND™ AP is automatically discharged, and insert the cartridge into the FREND™ System.

The testosterone result is based on the ratio of fluorescence by the FREND™ System at the FREND™ Testosterone cartridge Test and Reference zones. The magnitude of the fluorescent ratio is inversely proportional to the amount of testosterone in the sample, so a lower ratio of fluorescence correlated with a higher testosterone concentration.

Material provided

* Catalog number : FRTEAP 020

FREND™ Testosterone Cartridges	20
FREND™ Testosterone Pretreatment tubes	20
Disposable pipette tips	30
FREND™ Testosterone Code chip	01
FREND™ Testosterone package insert	01

One cartridge contains:

Testosterone	48.2 ± 48.2 ng
Monoclonal anti-testosterone antibody	32 ± 3.2 ng
Fluorescent particles	3.6 ± 0.36 ng

One pretreatment tube contains:

Gold antibody conjugates	30.0 ± 3.0 ng

Materials required but not provided

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

Warnings and precautions

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

- The FREND™ Testosterone cartridges are intended for *in vitro* diagnostic use only.
- Testosterone cartridges are only to be used on the FREND™ System.
- Testosterone cartridges are disposable, single use devices. Do not reuse them under any circumstances.
- Allow sealed cartridges to come to room temperature for approximately 15~30 minutes prior to use.
- Cartridges and Gold Antibody pretreatment tubes should not be frozen.
- Assure the humidity in the laboratory is in the 10~80% range and that the room temperature is in the range of 64~77°F (18~25°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 in the area used for cartridge storage.
- 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. Do not use the cartridges beyond the expiration date on the pouch.
- Do not use the cartridge and pretreatment tube if the pouch is damaged or the seal is broken.
- Perform testing as specified in the Package Insert Sheet and User Manual.
- Perform testing as specified in the package Insert and User manual. Keep the cartridge and pretreatment tube sealed in the pouch until ready for use.
- Use the cartridge immediately after opening the pouch.
- Handle specimens in accordance with the OSHA Standard on Blood borne Pathogens.
- 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 laboratory may be derived from human materials. Please use Universal Precautions when handling all specimens and controls. Wear disposable gloves when handling the cartridges and the samples.
- Do not ingest the silica gel packet found in the cartridge pouch.

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 eighteen months from the date of manufacture.

The expiration date is clearly indicated on the product box and the cartridges.

Materials	Cat. No.
Refrigerator temperature (2 ~ 8° C)	
Testosterone cartridges	FRTEAP 020
Testosterone pretreatment tubes	
Room temperature (18 ~ 25° C)	
Pipette Tips	None

Specimen collection and handling

Human serum or plasma (K_3 EDTA and lithium heparin) samples are acceptable for use with FREND™ Testosterone cartridges. 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 and then centrifuge for 10 minutes at 3,000 rpm.

For plasma (K_3 EDTA and lithium heparin), invert the sample ±five times immediately after collection to mix with anticoagulant. Centrifuge the sample for 10 minutes at 3,000 rpm. After centrifugation, separate the plasma from the packed cells.

Separated samples may be stored at 35~46°F (2~8°C) for up to 6 hours prior to analysis. Sample may be stored frozen at -4°F (-20°C) or below for use. Repeated freeze-thaw cycles should be avoided. Prior to assay, slowly bring frozen samples to room temperature 64~77°F (18~25°C) and gently but thoroughly mix before testing.

The sample required for the incubation step is 70 µL. After incubation, 35 µL of the incubated sample is pipetted onto the cartridge sample inlet automatically in the FREND™ AP and the cartridge is inserted into the instrument.

For optimal results, avoid grossly hemolytic, lipemic, or turbid specimens. Specimens should be free of aggregated fibrin, red blood cells, or other particulate matter. Sample containing particulate matter should be re-centrifuged before being tested.

When pipetting into the FREND™ Testosterone 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™ Testosterone Code chip included in each box of FREND™ Testosterone cartridges. The FREND™ Testosterone Code chip is specific for each lot of FREND™ Testosterone 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 Code chip into the Code chip slot at the rear of the FREND™ System following the arrows.
(2) Press the 'Setup' button on the 'Main' screen.
(3) Press the 'Code chip' button on the 'Setup' screen.
(4) The information embedded on the FREND™ Testosterone Code chip is automatically saved on the FREND™ System.
(5) When the Code chip installation is completed, press the 'OK' button to go to the 'Setup' screen.
(6) Press the 'Item' button on the 'Setup' screen.
(7) Check the FREND™ Testosterone cartridge lot number and the installation date of the Code chip.
(8) Press the 'Home' button to go to the 'Main' screen to begin running external quality control and patient samples.

Quality control

• FREND System QC cartridges

The FREND™ QC Cartridge contains multiple controls that check the optics of the system. By testing the QC Cartridge, (1) laser power (2) alignment, and (3) mechanical integrity components of the system are confirmed.

For each day of patient testing perform QC Cartridge testing. Refer to the quality control procedures section in the User Manual of the 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™ Testosterone test cartridge contains a built-in control feature. Fluorescence signal in the reference 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 reference zone signal is missing or lower than the threshold, the FREND™ System considers it an incorrect or failed test and produces an error message instead of a test result. In addition, with each cartridge run, the system monitors 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 Testosterone as a measure 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 according to the local requirements for each 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 a 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 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 no assay patient samples on the FREND™ System using the FREND™ Testosterone if quality control results do not fall within the acceptable ranges. Each laboratory operates under a different set of regulations. Every laboratory must follow the standardized procedures acceptable to the regulatory agencies to whom the laboratory is responsible.

• Specimen processing

- Preparation

Remove sufficient FREND™ Testosterone 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 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 previously from sample 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™ Testosterone cartridges, pretreatment tube and specimen at room temperature (18~25 ℃). Open the pouch and place FREND™ Testosterone 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

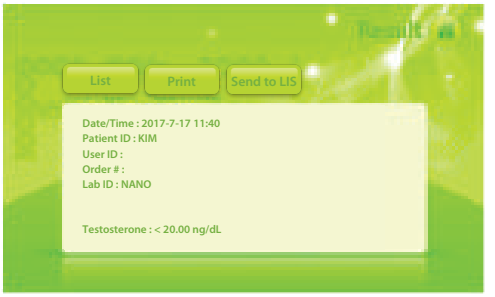
If the testosterone concentration of a specimen is found to be greater than the linearity limit of the assay (1,500 ng/dL) and a definitive result is required, the specimen should be diluted 1:2 with a low concentration sample that has been previously measure with the FREND™ Testosterone and then re-assayed according to the 'Assay Procedure'. Dilutions must be made manually and the final result of the diluted sample calculated manually by multiplying the result obtained on the diluted sample by the dilution factor.

※ Original unknown concentration of Testosterone
=(Concentration of diluted sample *2) – (concentration of low level sample)

Calculation or results

The FREND™ System performs all sample and reagent handling operations automatically within the cartridge once the sample has been loaded into the sample in let 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 is subtracted after which the net rate is automatically converted to Testosterone concentration in ng/dL based upon information stored on the FREND™ Code Testosterone Code chip.

Screen displayed for various concentration scenarios

Displayed result	Description
	Testosterone concentration Less than 20.00 ng/dL
	Testosterone concentration Not less than 20.00 ng/dL and not higher than 1,500 ng/dL
	Testosterone concentration Higher than 1,500 ng/dL

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™ Testosterone cartridge, is programmed to report 1500 ng/dL as the highest concentration of Testosterone measurable without dilution. The lowest measurable concentration is 20 ng/dL. If the result is below the lowest reportable range, it should be reported as such <20 ng/dL.

(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".2,3 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 Testosterone concentration determined with assays from different manufacturers can vary in a given sample due to differences in assay methods, calibration, and reagents.

(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™ Testosterone is to be used in licensed clinical laboratories with trained technologists and has not been evaluated in point-of-care settings.

(10) FREND™ Testosterone has not been evaluated in pediatric and adolescent (<21 years old) populations.

Performance characteristics

Precision

A single lot imprecision study was performed at the NanoEnTek laboratory as described in the CLSI protocol EP5-A3. Three serum pools were assayed for 20 days, 2 runs per day in duplicate using a single lot of FREND™ Testosterone reagent cartridge. The results are summarized below:

FREND™ Testosterone single site single lot precision

Sample	Mean Testosterone level (ng/dL)	Repeatability		Within-laboratory	
		SD	CV(%)	SD	CV(%)
1	39.723	4.451	11.2	4.692	11.8
2	202.965	16.670	8.2	17.296	8.5
3	1,012.208	54.748	5.4	57.480	5.7

Linearity

To demonstrate the linearity of the assay, a serum base pool with an elevated Testosterone (1,650 ng/dL) was prepared and diluted to a total of 7 levels according to the dilution protocol outlined in CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. At each dilution level, the samples were tested in duplicate to determine the experimental value of Testosterone. Linearity was demonstrated from <20 ng/dL to >1,500 ng/dL. The measuring range for the FREND™ Testosterone is 20 ~ 1,500 ng/dL.

Method comparison

Method comparison studies were performed in a CLIA-certified laboratory using de-identified fresh and frozen serum specimens. The reference method was the Architect 2nd generation Testosterone run on the Abbott ARCHITECT i System. All samples (157) analyzed in the clinical testing were split and tested by both ARCHITECT and the FREND™ Testosterone systems. Two samples were beyond the measuring range of one or both assay, leaving 155 samples for analysis. Passing-Bablok regression analysis gave a slope of 0.983, a y-intercept of -2.353 and a correlation(R) of 0.977. Comparability using CLSI guideline EP09-A3 shows that the two method compare favorably.

Matrix comparison

Matrix comparison study was performed according to CLSI EP14-A3. Testosterone concentration in 40 paired serum, lithium heparin plasma and K_3 EDTA plasma samples were measured using the FREND™ Testosterone. Passing-Bablok regression analyses of serum results (x) compared to lithium heparin plasma and K_3 EDTA result (y) yielded and acceptable regression (Slope=1.010, Intercept=-1.264, R^2=0.985 for heparinized plasma, Slope=1.006, Intercept=-8.186, R^2=0.987 for K_3 EDTA plasma respectively), indicating that FREND™ Testosterone can be measured equally well in serum, lithium heparin plasma, and K_3 EDTA plasma. It is not recommended to use the same specimen matrix when following patients because the results may not be interchangeable.

Reference interval

The normal range (reference interval) of testosterone measure in the FREND™ Testosterone Test System was established from 488 prospectively tested adult male and female individuals. The results, partitioned by gender and age range, are provided below.

Reference interval for the FREND Testosterone test system

Age range	Females	Males
21 ~ 49	<20~107.5	170.1~1,263.6
50 ~ 90	<20~150.3	152.4~1,095.2

Each laboratory should establish its own expected values for testosterone as performed on the FREND™ System.

Sensitivity

The Limit of Detection (LoD) for the FREND™ Testosterone was established according to the CLSI EP17-A2 protocol and was determined to be 14.3 ng/dL. The functional sensitivity was 19.66 ng/dL, and the measuring range was established at 20.0 ng/dL to 1500 ng/dL.

Specificity and Interferences

Interference was defined as recovery values outside of 10% of the known specimen mean concentration. Recovery within 90% to 110% of the expected testosterone was considered as lack of interference. The interference studies were performed as recommended in the CLSI EP07-A2 protocol. Results are summarized in the table below.

Interferent types	Interferent (concentration tested)	%Recovery Low testosterone	%Recovery High testosterone
Endogenous substances	Hemoglobin (500 mg/dL)	97.2	96.5
	Bilirubin conj. (30 mg/dL)	106.6	100.4
	Bilirubin unconj. (30 mg/dL)	99.6	98.9
	Triglyceride (3 g/dL)	99.6	94.4
	Total protein (12 g/dL)	96.5	100.8
	Biotin (30 ng/mL)	101.8	104.3
	SHBG (70 ng/mL)	99.9	100.2
HABA And RF	HAMA (70 ng/mL)	104.3	98.2
	Rheumatoid Factor (1,075 IU/mL)	103.8	100.2
Pharmaceuticals	Acetylcysteine (415 mg/mL)	97.9	99.4
	Ampicillin-Na (50.3 mg/mL)	98.7	100.6
	Ascorbic acid (60 mg/mL)	96.3	101.2
	Ca-Dohesilate (40 mg/mL)	101.3	101.5
	Cyclosporine (3 mg/mL)	97.4	96.8
	Cefoxitin (66 mg/mL)	101.1	97.9
	Heparin (3,000 U/L)	102.6	99.7
	Levodopa (4 mg/mL)	96.7	100.9
	Methyldopa (15 mg/mL)	101.0	97.1
	Metronidazole (120 mg/mL)	104.7	104.8
	Doxycycline (30 mg/mL)	100.5	97.9
	Acetylsalicylic acid (250 mg/mL)	94.7	98.4
	Rifampicin (640 mg/mL)	97.1	99.7
	Acetaminophen (200 mg/mL)	99.6	100.3
	Ibuprofen (250 mg/L)	95.7	97.4
	Theophylline (400 mg/mL)	102.1	99.5

Cross-reactivity

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

Cross-reactant	Cross-reactant concentration (ng/dL)	%Cross-reactivity	
		Low	High
Androstenedione (1,000 nmol/L)	28,641.0	0.0032	0.0337
Androsterone (1,000 nmol/L)	29,044.0	0.0004	0.0158
Cortisone (1,000 nmol/L)	36,044.0	0.0077	0.0300
Danazol (1,000 nmol/L)	33,746.0	0.0007	0.0496
Estradiol (200 nmol/L)	5,447.6	0.0033	0.5160
Estrone (500 nmol/L)	13,518.5	0.0144	0.0378
17-Ethinyl estradiol (1,00 ng/mL)	100,000.0	0.0033	0.0081
Progesterone (2,000 nmol/L)	62,892.0	0.0007	0.0167
Dexamethasone (5 µmol/L)	196,230.0	0.0255	0.0122
Ethisterone (20 nmol/L)	624.9	0.0437	0.3840
D(-) Norgestrel (20 ng/mL)	2,000.0	0.0125	0.1667
Prednisolone (2,000 nmol/L)	61,288.0	0.0001	0.0175
Prednisone (2,000 nmol/L)	71,686.0	0.0031	0.0040
Spironolactone (500 ng/mL)	50,000.0	0.0039	0.0172
Cortisol (10,000 nmol/L)	362,460.0	0.0004	0.0070
DHEA (50 nmol/L)	1,442.1	0.0178	1.0463
DHEAS (50 µmol/L)	1,842,500.0	0.0002	0.0009
Dihydrotestosterone (40 nmol/L)	1,161.7	0.2040	0.4134
Epitestosteone (100 nmol/L)	2,884.2	0.0467	0.1502
Ethynodiol diacetate (50 ng/mL)	5,000.0	0.0274	0.1280

References

(1) Burtis C.A. and Ashwood E.R., Ed. Tietz Textbook of Clinical Chemistry, 2nd Edition, W.B. Saunders Company. 1994, 1850-51.

(2) Dunn J.F., Nisula B.C. and Rodbard D. *Journal of Clinical Endocrinology and Metabolism*, 1981, 53:58-68.

(3) Boscato L.M., Stuard M.C. *Clinical Chemistry*. 1988, 27.

Glossary of symbols

	Caution, warning, Consult accompanying documents		*In vitro* diagnostic medical device
	Catalogue number / Reference number		Temperature limitation
	Lot number / Batch number		Contains sufficient for <n> tests
	Use by YYYY-MM-DD or YYYY-MM		Do not reuse
	Manufacturer		Do not use if package is damaged
	Authorized representative in the European Community		Medical prescription
	CE marking		Irritant

NanoEnTek

ivdst@nanoentek.com
www.nanoentek.com

Manufactured by

NanoEnTek, Inc.
851-14, Seohae-ro, Paltan-myeon, Hwaseong-si, Gyeonggi-do, 18531, Korea
Tel : +82-2-6220-7942 Fax : +82-2-6220-7999

EC REP

MT Promedt Consulting GmbH
Altenhofstrasse 80, 66386 St. Ingbert, Germany

NanoEnTek, Inc. (USA)
240 Bear Hill Road, Suite 101, Waltham, MA 02451, USA
Tel: +1-781-472-2558 Fax: +1-781-790-5649
www.nanoentek.com

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