1. INTENDED USE
The kit has been designed for the quantitative determination of Reverse Triiodothyronine (rT3) in human serum. The method can be used for samples over the range of 0-10ng/ml. The test has to be performed on the Maglumi fully auto analyzer (including Maglumi 1000, Maglumi 2000, Maglumi 2000 plus).

2. SUMMARY AND EXPLANATION OF THE TEST
Thyroxine (T4) is from the thyroid gland in the liver and kidney cells is peripherally converted into T3 and reverse T3 (rT3). T3 is the active hormone and is five times as potent as T4, but rT3 is almost biologically inactive. rT3 can also be included into the total thyroid screen on request or carried out separately. rT3 is primarily produced from monodeiodination of thyroxin in the peripheral tissue rather than by direct secretion by the thyroid gland. Physical, mental and environmental stresses can inhibit the deiodinating enzyme, causing less T4 to be converted to T3, thus decreasing the amount of active thyroid hormone available to the cells. More T4 is then shunted towards rT3 causing an elevation in rT3. Measuring rT3 levels is useful when ‘sick euthyroid’ conditions are suspected.

3. PRINCIPLE OF THE TEST
Competitive immunoluminometric assay:
Use an anti-rT3 polyclonal antibody to label FITC, and use a purified rT3 antigen to label ABEI. Sample, Calibrator, or Control, FITC Label, ABEI Label and nano magnetic microbeads coated with sheep anti-FITC are mixed thoroughly and incubated at 37°C, forming complexes; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of rT3 present in controls or samples.

4. KIT COMPONENTS
4.1 Material supplies
<table>
<thead>
<tr>
<th>Reagent Integral for 100 determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaN3, coated with sheep anti-FITC polyclonal antibody.</td>
</tr>
<tr>
<td>Calibrator low</td>
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<tr>
<td>Calibrator high</td>
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<tr>
<td>ABEI Label: rT3 monoclonal antibody labeled ABEI, containing BSA, 0.2%NaN3.</td>
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<tr>
<td>FITC Label: purified anti-rT3 antigen labeled FITC, containing BSA, 0.2%NaN3.</td>
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</tbody>
</table>

All reagents are provided ready-to-use.

Accessories required but not provided
- Maglumi Reaction module
- Maglumi Starter kit 1+2
- Maglumi Light check
- Maglumi Wash/System Liquid

4.2 Preparation of the Reagent Integral
Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation!) Remove the sealing and turn the small wheel of the magnetic microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 mins. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

Do not interchange Nano Magnetic Microbeads from different reagents!

4.3 Storage of the Reagents Integral
- Sealed: Stored at 2-8°C until the expiry date.
- Opened: Stable for 4 weeks. After this period, it is still possible to keep on using the Reagent Integral provided that the controls are found within the expected ranges.
- Keep upright for storage.
- Keep away from direct sunlight.

5. Origin of Calibrators.
Calibrators in the Reagent Kit are from Sigma.
Biological risk: synthetic materials, processed by HPLC purification, with a...
6. Calibration

6.1 2 point recalibration
Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each recalibration.

6.2 Frequency of Recalibration
- After each exchange of lot (Reagent Integral or Starter Reagents)
- Every week and/or each time a new Integral is used
- After each servicing of the Maglumi Fully Auto analyzer.
- If controls are beyond the expected range.

7. Sample Collection, Material and Storage
- Collect samples using standard procedures.
- Sample material: serum.
- Store at 2-8°C: 24 hours.
- For longer storage periods: freeze to below -20°C.
- Avoid repeated freezing and thawing cycles.
- Stored samples should be thoroughly mixed prior to use (Vortex mixer).

8. WARNING AND PRECAUTIONS FOR USERS
- For use in IN-VITRO diagnostic procedures only.
- Do not interchange reagents from different lots. Do not use kit components beyond their labeled expiry date.
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach (USP 24,2000,p.2143).
- A minimum of one hour at 121°C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
- The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent, these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.

9. Test Procedure
To ensure proper test performance, strictly adhere to the operating instructions of the Maglumi Fully Auto Analyzer. Each test parameter is identified via a barcode on the Reagent Integral. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

| 50μl | 15 min | 400μl each time | Sample, calibrator or controls |
| 180μl | Incubation | Measurement |
| 20μl | | |
| | | | ABCI Label |
| | | | FIC Label |
| | | | Nano magnetic microbeads |

10. Quality Control
- Observe quality control guidelines for medical laboratories.
- Use suitable controls for in-house quality control.

11 Results
11.1 Calculation of Results
- The analyzer automatically calculates the rT3 concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/ml. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

11.2 Interpretation of Results
- Reference values: 0.31–0.95 ng/ml.
- Results may differ between laboratories due to variations in population and test method. Each laboratory should establish its own reference range.

12. Limitations of the procedure
12.1 Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results. Procedural directions must be followed exactly and careful technique must be used to obtain valid results. Any modification of the procedure is likely to alter the results. Bacterial contamination or repeated freeze-thaw cycles may affect the test results.

12.2 HAMA
Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralising agents are added, extremely high HAMA serum concentrations may occasionally influence results.

13. Performance Characteristics
13.1 Accuracy
Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratios with diluents, and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90%-110%.

13.2 Precision
Intra-assay coefficient of variation was evaluated on Calibrator High repeatedly measured 10 times in the same assay, calculating their coefficient of variation, the results should <10%. Inter-assay coefficient of variation was evaluated on three batches of kit, repeatedly measured 10 times of Calibrator High, calculating three batches of kit for Calibrator High between the measured values of the coefficients of variation, the results should <15%.

13.3 Sensitivity
The sensitivity is defined as the concentration of rT3 equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 0.05ng/ml.

13.4 Specificity
The results about T3,T4 standard concentration should accord with the following description.
When T3<10ng/ml, the detection results of rT3 <1.0ng/ml; When T4 >300ng/ml, the detection results of rT3 <1.0ng/ml.

13.5 Linearity
Conduct a logarithmic transform to the RLU value and concentration value of each standard. After a double logarithmic fitting, the absolute value of its linearity should exceed 0.9800.

14. References
3. Serum 3,3’,5'-Triiodothyronine (rT3) and 3,3’,5'-Triiodothyronine/rT3 Are Prognostic Markers in Critically Ill Patients and Are Associated with Postmortem Tissue Deiodinase Activities. The Journal of Clinical Endocrinology & Metabolism Vol. 90, No. 8 4559-4565
4. Clinical significance of serum reverse T3 analysis in endocrine tests of the thyroid-parathyroid system