ELISA kits available from ADI (see details at the web site)

#0010  Human Leptin
#200-120-AGH  Human globular Adiponectin (gAcrp30)
#0700  Human Sex Hormone Binding Glob (SHBG)
#0900  Human IGF-Binding Protein 1 (IGFBP1)
#1000  Human C-Reactive Protein (CRP)
#100-110-RSH  Human Resistin /FIZZ3
#100-140-ADH  Human Adiponectin (Acrp30)
#100-160-ANH  Human Angiogenin
#100-180-APH  Human Angiopoietin-2 (Ang-2)
#100-190-B7H  Human Bone Morphogenic Protein 7 (BMP-7)
#1190  Human Serum Albumin  #1200  Human Albumin (Urinary)
#1750  Human IgG (total)  #1760  Human IgM
#1800  Human IgE  #1810  Human Ferritin
#1210  Human Transferrin (Tf)  #0020  Beta-2 microglobulin
#1600  Human Growth Hormone (GH)

#0060  Human Pancreatic Colorectal cancer (CA-242)
#1820  Human Ovarian Cancer (CA125)  #1830  Human CA153
#1840  Human Pancreatic & GI Cancer (CA199)
#1310  Human Pancreatic Lipase
#1400  Human Prostatic Acid Phosphatase (PAP)
#1500  Human Prostate Specific Antigen (PSA)  #1510  free PSA (fPSA)
#0500  Human Alpha Fetoprotein (AFP)
#0050  Human Neuron Specific Enolase (NSE)

#0030  Human Insulin  #0040  Human C-peptide
#0100  Human Luteinizing Hormone (LH)
#0200  Human Folicle Stimulating Hormone (FSH)
#0300  Human Prolactin (PRL)
#0400  Human Chorionic Gonadotropin (HCG)  #0410  HCG-free beta

#0600  Human Thyroid Stimulating Hormone (TSH)
#1100  Human Total Thyroxine (T4)  #1110  Human Free T4 (fT4)
#1650  Human free triiodothyronine (fT3)  #1700  Human T3 (toal)

#1850  Human Cortisol  #1860  Human Progesterone
#1865  Human Pregnolone  #1875  Human Aldosterone
#1880  Human Testosterone  #1885  Human free Testosterone
#1910  Human Androstenedione  #1920  Human Estradiol
#1925  Human Estrone  #1940  Dihydrotestosterone (DHT)
#1950  Human DHEA-sulphate (DHEA-S)
#3400  Human serum Neopterin

#3000  Human Rheumatoid Factors IgM (RF)
#3100  Human anti-dsDNA
#3200  Anti-Nuclear Antibodies (ANA)

Rheumatoid Factor IgM (RF IgM)

ELISA KIT  Cat. No. 3000

For Quantitative Determination of RF IgM
In Human Serum

For In Vitro Research Use Only

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777
Email: service@4adi.com
Web Site: www.4adi.com
PERFORMANCE CHARACTERISTICS

PRECISION

<table>
<thead>
<tr>
<th>Intra-assay precision:</th>
<th>Sample</th>
<th>Mean (IU/ml)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29.2</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>116.3</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>294.4</td>
<td>4.9</td>
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<table>
<thead>
<tr>
<th>Inter-assay precision:</th>
<th>Sample</th>
<th>Mean (IU/ml)</th>
<th>CV%</th>
</tr>
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<tbody>
<tr>
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<td>26.7</td>
<td>4.0</td>
<td></td>
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<tr>
<td>2</td>
<td>102.0</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>275.1</td>
<td>3.2</td>
<td></td>
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</table>

EXPECTED VALUES

It is recommended that each laboratory must determine its own negative and positive values. Samples containing less than 25 IU/ml RF IgM can be considered as RF IgM-negative; samples showing greater than 25 IU/ml concentrations can be considered as RF IgM-positive. Concentrations of higher than 75 IU/ml RF IgM usually indicate rheumatoid arthritis. RF IgM are found in about 2-10% of apparently healthy Caucasian adults and in about 50-70% of adults with classical rheumatoid arthritis.

Clinical Study

A clinical study using the ADI's RF IgM ELISA was conducted and results are summarized below:

<table>
<thead>
<tr>
<th>ADI</th>
<th>Commercial ELISA</th>
<th>Commercial Latex test</th>
</tr>
</thead>
<tbody>
<tr>
<td>N 69</td>
<td>69</td>
<td>69</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt;25 IU/ml</td>
<td>29</td>
</tr>
<tr>
<td>Range</td>
<td>&lt;20 IU/ml</td>
<td>15</td>
</tr>
<tr>
<td>Positive</td>
<td>25-75 IU/ml</td>
<td>15</td>
</tr>
<tr>
<td>Range</td>
<td>20-60 IU/ml</td>
<td>&gt;1:20</td>
</tr>
<tr>
<td>Rheum. Arth.</td>
<td>&gt;75 IU/ml</td>
<td>&gt;60 IU/ml</td>
</tr>
</tbody>
</table>

Species Crossreactivity

This kit is recommended for human samples only. Its utility in other species such as mouse, rat, or monkey etc has not been tested. ADI has a separate RF ELISA kit for mouse samples (#6200).

References:
WORKSHEET OF TYPICAL ASSAY

<table>
<thead>
<tr>
<th>Wells</th>
<th>Stds (IU/ml)</th>
<th>Mean A_{450} nm</th>
<th>Calcul. Conc. (IU/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>0</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>B1, B2</td>
<td>15</td>
<td>0.223</td>
<td></td>
</tr>
<tr>
<td>C1, C2</td>
<td>50</td>
<td>0.550</td>
<td></td>
</tr>
<tr>
<td>D1, D2</td>
<td>150</td>
<td>1.222</td>
<td></td>
</tr>
<tr>
<td>E1, E2</td>
<td>500</td>
<td>2.154</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: These data are for demonstration purpose only. A complete set of negative, positive, and calibrator standards set must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the sample diluents from the mean absorbance values of negative & positive controls, calibrator, and samples. The RF IgM values for the patient samples can be calculated as follows:

\[
\text{IU/ml of sample} = \frac{\text{Net absorbance of test sample}}{\text{Net absorbance of the calibrator}} \times \text{IU/ml of calibrator}
\]

PRINCIPLE OF THE TEST

Rheumatoid Factor IgM (RF IgM) ELISA kit is based on binding of RF IgM from serum samples to human gamma globulin immobilized on microtiter wells. After a washing step, anti-human IgM-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of RF IgM present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of RF IgM in samples is calculated on the basis of the absorbance of the negative, positive, and, calibrator controls.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Rheumatoid Factor IgM ELISA Kit is intended for in vitro research use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Negative, Positive, and Calibrator controls have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed , these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

REAGENTS PREPARATIONS:

Wash buffer is supplied as 50x stock. Dilute 20 ml into 980 ml de-ionized or distilled water, mix, and store at room temp for 1-2 weeks. It can be stored at 4oC for long term storage.

Sample Diluent (5X): Dilute 20 ml into 80 ml de-ionized or distilled water.

Dilute serum sample 1:100 in 1x sample diluent (5 ul sample in 495 ul buffer).

Alpha Diagnostic Intl. (www.4adi.com) 3000/IB120229A  
Page 2
STORAGE AND STABILITY
The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Label or mark the microtiter well strips to be used on the plate. Dilute controls, calibrators, and serum samples 1:100 (5 µl of sample in a total volume of 500 µl of sample diluents). Dilute wash buffer (1:50) with distilled water (20 ml stock in total of 1-liter). Dilute Sample Diluent (5X): Dilute 20 ml into 80 ml de-ionized or distilled water. Standards and controls are supplied pre-diluted.

1. Pipet 100 µl of diluted sample diluents, negative & positive controls, calibrator, and diluted serum samples into appropriate wells in duplicate. Cover the plate and incubate for 30 minutes at room temperature (20-28oC).

2. Aspirate and wash the wells 3 times with 300 µl of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

3. Add 100 µl of antibody-enzyme conjugate into each well. Mix gently. Cover the plate and incubate for 15 minutes at room temperature (20-28oC).

4. Aspirate and wash the wells 3 times with 300 µl of diluted wash buffer, as above.

5. Dispense 100 ul TMB substrate per well. Mix the plate gently for 5-10 seconds. Cover the plate and incubate for 15 minutes at room temperature. Blue color develops into standards and positive samples.

6. Stop the reaction by adding 100 µl of stopping solution to all wells at the same timed intervals as in step 8. Mix gently. Blue color turns yellow.

7. Measure the absorbance at 450 nm using an ELISA reader.

NOTES
Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of negative & positive standards and calibrator on each plate. The unused strips should be stored in a sealed bag at 4°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

QUALITY CONTROL OF THE TEST
1. OD values will vary with the temperature and length of incubation.
2. The O.D. of the Standards A (reagents blank) should be <0.250 and Standard E >1.100.
3. The value of positive and negative controls should be within the range of indicated value.

Calculation of results
For Rheumatoid Factor IgM a 4-Parameter-Fit with lin-log or lin-lin coordinates for optical density and concentration is the data reduction method of choice.

Recommended Lin-Log Plot
First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Interpretation of results
In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Rheumatoid Factor IgM test:

<table>
<thead>
<tr>
<th>Rheumatoid Factor IgM [IU/ml]</th>
<th>Normal</th>
<th>Elevated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 20</td>
<td>&gt; 20</td>
</tr>
</tbody>
</table>

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum Rheumatoid Factor.

LIMITATIONS OF PROCEDURE
The absence of rheumatoid factor does not rule out rheumatoid arthritis. Rheumatoid Factor may appear transiently during various infections. The Rheumatoid Factor IgM ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.