Elevated AFP concentrations in maternal serum or during pregnancy can indicate spina bifida and anencephaly. Measurement of AFP makes a contribution to the risk assessment for trisomy 21 (Down syndrome) in the second trimester of pregnancy together with hCG-B and other parameters, such as exact gestational age and maternal weight. In a trisomy 21 affected pregnancy the maternal serum concentration of AFP is decreased whereas the maternal serum hCG-B concentration is approximately twice the normal median. The risk for a trisomy 21 affected pregnancy in the second trimester can be calculated by a suitable software (see Materials required, but not provided) using the algorithm as described by Cuckle et al. and the respective assay specific parameters.

**Test principle**

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 6 µL of sample, a biotinylated monoclonal AFP-specific antibody, and a monoclonal AFP-specific antibody labeled with a ruthenium complex react to form a sandwich complex.

- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the cobas link.

**Reagents - working solutions**

The cobas e pack is labeled as AFP.

**M Streptavidin-coated microparticles, 1 bottle, 14.1 mL:**

Streptavidin-coated microparticles 0.72 mg/mL; preservative.

**R1 Anti-AFP-Ab-biotin, 1 bottle, 19.7 mL:**

Biotinylated monoclonal anti-AFP antibodies (mouse) 4.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

**R2 Anti-AFP-Ab-Ruby[1] 1 bottle, 19.7 mL:**

Monoclonal anti-AFP antibodies (mouse) labeled with ruthenium complex 12.0 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

**Precautions and warnings**

For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request. Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

**Reagent handling**

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the cobas link.

**Storage and stability**

Store at 2-8 °C. Do not freeze. Store the cobas e pack upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

**Stability:**

<table>
<thead>
<tr>
<th>Unopened at 2-8 °C</th>
<th>Up to the stated expiration date on the cobas e 801 analyzer</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 weeks</td>
<td></td>
</tr>
</tbody>
</table>

**Specimen collection and preparation**

Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes or tubes containing separating gel.
Elecsys AFP

Li-heparin, K₂-EDTA and K₃-EDTA plasma.

Plasma tubes containing separating gel can be used.

**Criterion:** Slope 0.9-1.1 + intercept within ± 1.5 IU/mL + coefficient of correlation ≥ 0.95.

Stable for 5 days at 20-25 °C, 14 days at 2-8 °C, 6 months at -20 °C (± 5 °C). The samples may be frozen 3 times.

The suitability of plasma samples for estimating the risk of trisomy 21 has not been evaluated.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

**Materials provided**

See “Reagents – working solutions” section for reagents.

**Materials required (but not provided)**

- 04487761190, AFP CalSet II, for 4 x 1.0 mL
- 11776452122, PreciControl Tumor Marker, for 4 x 3.0 mL or
- 11731416190, PreciControl Universal, for 4 x 3.0 mL
- 07299001190, Diluent Universal, 45.2 mL sample diluent
- General laboratory equipment
- cobas e 801 analyzer

Accessories for the cobas e 801 analyzer:

- 06908799190, ProCell II M, 2 x 2 L system solution
- 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- 07484540901, Reservoir Cups, 8 cups to supply ProCell II M and CleanCell M
- 06908853190, ProCell II M, 2 x 2 L wash solution
- 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- 074845425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply SE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- 074845433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply SE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- 11298500316, ISE Cleaning Solution/Elecsys SysClean, 3 x 100 mL system cleaning solution

For risk calculation of trisomy 21:

- A suitable software, e.g. 05126193, SedwLab (V5.0 or later), single user licence
  05195047, SedwLab (V5.0 or later), multi user licence
- 03271749190, HCG+ß, 100 tests
- 07251025190, Elecsys HCG+ß, 300 tests
- 03302652190, HCG+ß CalSet, for 4 x 1.0 mL

**Assay**

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) cobas e pack on the reagent manager. Avoid foam formation. The system automatically regulates the pressure of the reagents and the opening/closing of the cobas e pack.

**Calibration**

Traceability: This method has been standardized against the 1st IRP WHO Reference Standard 72/2225.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

**Calibration frequency:** Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the cobas e pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same cobas e pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

**Quality control**

For quality control, use PreciControl Tumor Marker or PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per cobas e pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

**Calculation**

The analyzer automatically calculates the analyte concentration of each sample either in IU/mL, ng/mL, kIU/L or additionally in IU/L

Conversion factors:

\[ \text{IU/mL} \times 1.21 = \text{ng/mL} \]

\[ \text{ng/mL} \times 0.83 = \text{IU/mL} \]

**Limitations - interference**

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed. **Endogenous substances**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>≤ 1112 μmol/L or ≤ 65 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>≤ 1.37 mmol/L or ≤ 2200 mg/dL</td>
</tr>
<tr>
<td>Intralipid</td>
<td>≤ 1500 mg/dL</td>
</tr>
<tr>
<td>Biotin</td>
<td>≤ 738 nmol/L or ≤ 180 ng/mL</td>
</tr>
<tr>
<td>Rheumatoid factors</td>
<td>≤ 1500 IU/mL</td>
</tr>
</tbody>
</table>

Criterion: Recovery of ± 0.4 IU/mL of initial value ≤ 4 IU/mL and ± 10 % of initial value > 4 IU/mL.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

There is no high-dose hook effect at AFP concentrations up to 1 million IU/mL (1.21 million ng/mL).

**Pharmaceutical substances**

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special cancer drugs were tested. No interference with the assay was found.
Elecsys AFP

Special cancer drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration tested (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>75</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>1000</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>225</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>500</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>1000</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>50</td>
</tr>
<tr>
<td>Mitomycin</td>
<td>25</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>1000</td>
</tr>
<tr>
<td>Etoposide</td>
<td>400</td>
</tr>
<tr>
<td>Taxol</td>
<td>5.5</td>
</tr>
</tbody>
</table>

In rare cases, interference due to extremely high titters of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.75-100 IU/mL or 0.908-1210 ng/mL (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.75 IU/mL or < 0.908 ng/mL. Values above the measuring range are reported as > 1000 IU/mL or > 1210 ng/mL (or up to 50000 IU/mL or 60500 ng/mL for 50-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.75 IU/mL
Limit of Detection = 1.5 IU/mL
Limit of Quantitation = 2.25 IU/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

An internal study was performed based on guidance from the CLSI, protocol EP17-A2. Limit of Blank and Limit of Detection were determined to be the following:

Limit of Blank = 0.614 IU/mL
Limit of Detection = 0.712 IU/mL

For Limit of Quantitation ≥ 4 human serum samples were measured over 5 days with 5 replicates per day on one analyzer. With an intermediate precision CV of ≤ 20 %, the Limit of Quantitation was 1.05 IU/mL.

Dilution

Samples with AFP concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:50 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 20 IU/mL (> 24.2 ng/mL).

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzer, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

Results of following studies using the Elecsys AFP assay see below:


Following AFP values were found in serum samples from 646 healthy test subjects:

≤ 5.8 IU/mL or ≤ 7.0 ng/mL for 95 % of the results.

AFP median values for completed weeks of pregnancy (defined as completed weeks of pregnancy beginning with the start of the last menstruation phase):

<table>
<thead>
<tr>
<th>Weeks</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>382</td>
<td>1782</td>
<td>2386</td>
<td>353</td>
<td>146</td>
</tr>
<tr>
<td>IU/mL</td>
<td>23.2</td>
<td>25.6</td>
<td>30.0</td>
<td>33.5</td>
<td>40.1</td>
</tr>
<tr>
<td>ng/mL</td>
<td>27.9</td>
<td>30.9</td>
<td>36.1</td>
<td>40.4</td>
<td>48.3</td>
</tr>
</tbody>
</table>

b) Multicenter study to determine reference values for evaluating the risk of trisomy 21 in maternal serum (study No. BO1P019, status March 2003).

Values from serum samples of 1753 pregnant women in total (relevant gestational weeks 14 to 18) were evaluated.

Measurements with the Elecsys HCG-B assay and the Elecsys AFP assay were conducted in 5 clinical centers in Belgium, France, and Germany.

The gestational age in days determined by ultrasound was given for each sample. From a log-linear regression analysis of all 1753 AFP values versus gestational age the following median values were calculated for the middle of the respective weeks (e.g. week 14 + 3 days):

<table>
<thead>
<tr>
<th>Weeks</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>IU/mL</td>
<td>20.9</td>
<td>24.0</td>
<td>27.6</td>
<td>31.7</td>
<td>36.4</td>
</tr>
<tr>
<td>ng/mL</td>
<td>25.3</td>
<td>29.0</td>
<td>33.3</td>
<td>38.3</td>
<td>44.0</td>
</tr>
</tbody>
</table>

Note: For prenatal testing it is recommended that the median values be re-evaluated periodically (1 to 3 years) and whenever methodology changes. The transferability of the reference values to plasma samples has not been verified.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzer is given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-AS) of the CLSI (Clinical and Laboratory Standards Institute); 2 runs per day in duplicate each for 21 days (n = 84).

The following results were obtained:

<table>
<thead>
<tr>
<th>cobas e 801 analyzer</th>
<th>Repeatability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample</td>
</tr>
<tr>
<td></td>
<td>IU/mL</td>
</tr>
<tr>
<td>Human serum 1</td>
<td>1.87</td>
</tr>
<tr>
<td>Human serum 2</td>
<td>5.34</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>46.1</td>
</tr>
<tr>
<td>Human serum 4</td>
<td>485</td>
</tr>
<tr>
<td>Human serum 5</td>
<td>935</td>
</tr>
<tr>
<td>PC1 Tumor Marker 1</td>
<td>8.71</td>
</tr>
<tr>
<td>PC Tumor Marker 2</td>
<td>87.8</td>
</tr>
<tr>
<td>PC Universal 1</td>
<td>8.80</td>
</tr>
<tr>
<td>PC Universal 2</td>
<td>46.2</td>
</tr>
</tbody>
</table>

b) PC = PreciControl
## Elecsys AFP

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intermediate precision</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>CV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human serum 1</td>
<td>1.87</td>
<td>2.28</td>
<td>0.040</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>Human serum 2</td>
<td>5.34</td>
<td>6.46</td>
<td>0.102</td>
<td>0.123</td>
<td></td>
</tr>
<tr>
<td>Human serum 3</td>
<td>46.1</td>
<td>55.8</td>
<td>0.840</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td>Human serum 4</td>
<td>485</td>
<td>587</td>
<td>7.66</td>
<td>9.27</td>
<td></td>
</tr>
<tr>
<td>Human serum 5</td>
<td>935</td>
<td>1131</td>
<td>18.7</td>
<td>22.6</td>
<td></td>
</tr>
<tr>
<td>PC Tumor Marker1</td>
<td>8.71</td>
<td>10.5</td>
<td>0.154</td>
<td>0.186</td>
<td></td>
</tr>
<tr>
<td>PC Tumor Marker2</td>
<td>87.8</td>
<td>106</td>
<td>1.27</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>PC Univera1</td>
<td>8.80</td>
<td>10.6</td>
<td>0.136</td>
<td>0.165</td>
<td></td>
</tr>
<tr>
<td>PC Univera2</td>
<td>46.2</td>
<td>55</td>
<td>0.741</td>
<td>0.897</td>
<td></td>
</tr>
</tbody>
</table>

### Method comparison

A comparison of the Elecsys AFP assay, [Ref 07026706190 (cobas e 801 analyzer; y) with the Elecsys AFP assay, [Ref 04481798190 (cobas e 801 analyzer; x) gave the following correlations (IU/mL): Number of serum samples measured: 165

<table>
<thead>
<tr>
<th></th>
<th>Passing/Bablok</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Linear regression</td>
</tr>
<tr>
<td>y = 0.961x - 0.106</td>
<td>y = 0.964x - 0.589</td>
</tr>
<tr>
<td>τ = 0.980</td>
<td>r = 0.999</td>
</tr>
</tbody>
</table>

The sample concentrations were between 0.783 and 954 IU/mL.

### References


For further information, please refer to the appropriate operator’s manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

- **CONTENT**
- **SYSTEM**
- **REAGENT**
- **CALIBRATOR**
- **DTIN**

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