Contents of the kit

1. One bottle of TRACER (11 mL), ready to use, containing < 980 kBq 125I-anti-CA125 antibody and biotin-capture antibody in buffer with red dye and 0.1 % NaN3.

2. One bottle (10 mL) of STANDARD ZERO (S0) in aqueous solution with 0.1 % NaN3.

3. Five vials of STANDARDS S1-S5 (5 x 1 mL), containing 15-30-80-200-500 U/mL CA125 in human serum with 0.1 % NaN3. Assay calibration was performed using Fujirebio Diagnostics Inc. CA125HI RIA.

4. Two vials of CONTROL SERUM (1 mL) containing app. 50 U/mL and 100 U/mL CA125 in human serum with 0.1 % NaN3. The concentrations of controls are specified in the quality certificate enclosed.

5. COATED TUBES, ready to use.

   - Reactive test tubes, 12x75 mm, packed in plastic boxes. (RK-125CT: 2 boxes, 2x50 pcs; RK-125CT50: 1 box, 1x50 pcs).

6. One bottle of WASH BUFFER CONCENTRATE (20 mL), containing 0.2% NaN3. See Preparation of reagents.

   Quality certificate

Pack leaflet

Materials, tools and equipment required

- common laboratory equipment
- 100 µl precision micropipette
- 100 µl repeating pipette
- 2000 µl repeating pipette or dispenser
- horizontal shaker (at least 600 rpm)
- plastic foil to cover tubes
- absorbent tissue
- gamma-counter with software

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Hemolyzed and lipemic specimens may give false values and should be avoided.

Samples with a CA125 concentration higher than 500 U/mL should be diluted with S0 zero standard and reassayed. Recommended dilution: 10-fold (450 µL S0 + 50 µL sample).

Preparation of reagents, storage

Store the reagents between 2-8°C after opening. At this temperature each reagent is stable until the expiration date of the kit. The actual expiration date is given on the package label and in the quality certificate.

Add the wash buffer concentrate (20 mL) to 700 mL distilled water to obtain 720 mL wash solution. After dilution, store at 2-8°C until the expiration date of the kit.

CAUTION! Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Optional protocol

An incubation time of 60 minutes can be used. Typical cpm outputs when using fresh and old tracer are shown below:

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Standard</th>
<th>Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decant the fluid and blot on filter paper</td>
<td>2000</td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>Decant the fluid and blot on filter paper</td>
<td>2000</td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>Decant the fluid and blot on filter paper</td>
<td>2000</td>
<td>2000</td>
<td>2000</td>
</tr>
</tbody>
</table>

Count radioactivity (60 sec/tube) Calculate the results

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

<table>
<thead>
<tr>
<th>tracer</th>
<th>old</th>
<th>fresh</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>206237</td>
<td>210354</td>
</tr>
<tr>
<td>S0</td>
<td>584</td>
<td>560</td>
</tr>
<tr>
<td>S1</td>
<td>2038</td>
<td>1607</td>
</tr>
<tr>
<td>S2</td>
<td>3358</td>
<td>2769</td>
</tr>
<tr>
<td>S3</td>
<td>7744</td>
<td>6548</td>
</tr>
<tr>
<td>S4</td>
<td>17810</td>
<td>16225</td>
</tr>
<tr>
<td>S5</td>
<td>42288</td>
<td>35869</td>
</tr>
</tbody>
</table>

[Reference]: Fujirebio Diagnostics Inc. antibodies
**Calculation of results**

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2. Calculate the average count per minute (CPM) for each pair of assay tubes. Calculate the normalized percent binding for each standard, control, and sample respectively by using the following equation:

\[ \text{B/T}(\%) = \frac{S_{i}/C - T}{S_{0}/C - T} \times 100 \]

Using semi-logarithmic graph paper plot the B/T(%) for each standard versus the corresponding concentration of CA125. Determine the CA125 concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range. Out of fitting programs applied for computerized data processing, spline fittings are recommended.

**Table 2. Typical assay data**

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Mean cpm</th>
<th>B/T%</th>
<th>CA125 U/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>291557</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S0</td>
<td>278</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>2177</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>3610</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>8361</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>20729</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>S5</td>
<td>46876</td>
<td>16.1</td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>5642</td>
<td>1.9</td>
<td>51.7</td>
</tr>
<tr>
<td>CII</td>
<td>10973</td>
<td>3.8</td>
<td>105.9</td>
</tr>
</tbody>
</table>

**Performance characteristics**

**Specificity**

The antibodies used in this assay guarantee a measurement completely specific for CA125.

**Sensitivity**

Based on 120 determinations, with 60 blank and 60 low-level samples and with 95% probability, measurement limits are:
- Limit of Blank (LoB): 0.56 U/mL
- Limit of Detection (LoD): 1.08 U/mL

For results under LoB, should report as "analyte not detected". For results between LoB and LoD, should report as "analyte detected", concentration < 1.08 U/mL.

**Precision and reproducibility**

Four serum pools were assayed in 20 replicates to determine intra-assay precision. To determine inter-assay precision they were measured in duplicates in 20 independent assays. Values obtained are shown below.

<table>
<thead>
<tr>
<th>Intra-assay</th>
<th>Inter-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (U/mL)</td>
<td>CV%</td>
</tr>
<tr>
<td>24.39</td>
<td>9.99</td>
</tr>
<tr>
<td>24.31</td>
<td>5.71</td>
</tr>
<tr>
<td>44.26</td>
<td>1.42</td>
</tr>
<tr>
<td>44.67</td>
<td>3.11</td>
</tr>
<tr>
<td>154.59</td>
<td>2.31</td>
</tr>
<tr>
<td>157.0</td>
<td>3.12</td>
</tr>
<tr>
<td>305.87</td>
<td>1.98</td>
</tr>
<tr>
<td>316.42</td>
<td>2.80</td>
</tr>
</tbody>
</table>

**Linearity – dilution test**

Six individual serum samples were serially diluted with zero-standard and measured according to kit protocol. Mean recovery after dilution was 92.6%. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

\[ Y = 0.9899X - 4.2676 \quad R^2 = 0.9967 \quad n = 20 \]

**Recovery**

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of CA125. The average per cent recovery for 3 serum samples spiked with CA125 at 3 levels each was 103.37%, with a range of 99% to 107%.

**Hook effect**

No hook effect is observed for concentrations lower than 25000 U/mL.

**Expected Values**

It is recommended that each laboratory determine a reference range for its own patient population. Serum samples from 408 presumably healthy, non-pregnant female blood donors were evaluated:

- Samples < 35 U/mL: 386 (94.6%)
- Samples < 55 U/mL: 404 (99.0%)

**Procedural notes**

- The non-respect of the instructions in this insert may affect results significantly.
- Components from various lots or from kits of different manufacturers should not be mixed or interchanged.
- Source of error! To ensure the efficient rotation, tubes should be firmly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

**Limitations**

- The CA125 assay should not be used as a cancer screening test.
- CA125 assay values greater than or equal to 35 U/mL can be found in some healthy individuals and in patients with non-malignant conditions.
- A CA125 value below 35 U/mL does not indicate the absence of residual ovarian cancer.
- Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other diagnostic procedures.
- Specimens from patients who have received mouse immunoglobulin for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Serum from such individuals may produce erroneous results.

**Precautions**

**Radioactivity**

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

**Biohazard**

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunonassay), and were found to be negative for the presence of antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody and Hepatitis-B surface Antigen (HBsAg). Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as potentially infectious materials.

All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as potentially infectious materials.

**Chemical hazard**

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 68 mg.

**Storage and shelf life**

Store this product at a temperature of 2-8°C. Shelf-life: 60 days from availability.

**Use by**

- CONTROL
- Control

**Batch code**

- CAL
- Standard

**Caution, consult accompanying documents**

- CT
- Coated tube

**Biological risk**

- TRAC
- Tracer

**Consult operating instructions**

- WASI
- Wash buffer

**In vitro diagnostic medical device**

- M
- 2-8°C

**Manufacturer Catalogue number**

- Radioactive material

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**Legal note**

CA125™ is a trade mark of Fujirebio Diagnostics Inc. (FDI). The present CA125 IRMA is based on the use of the OC125 and M11 antibodies, which are available exclusively through FDI, and its licensed distributors.

**Updated:** April/2016