# Unconjugated Estriol [$^{125}$I] RIA KIT

(REF: RK-3CT)

The unconjugated Estriol [$^{125}$I] RIA kit is designed fot the quantitative *in vitro* determination of unconjugated estriol in human serum, in the range of 0-120 nmol/l (0-34.6 ng/ml).

## Introduction

Estriol is one of the most important steroid hormones in women. Estriol is almost exclusively produced during pregnancy, originating from a precursor synthesized by fetal adrenal glands and then converted to estriol by fetal liver and placenta.

In the maternal liver, estriol is conjugated to form sulphates and glucoronides, which are then excreted in the urine with a half-life of app. 20 minutes. In the maternal circulation unconjugated estriol accounts for app. 10% of total estriol.

Unconjugated estriol concentration rises during pregnancy, being 2 - 3 orders of magnitude higher in the final trimester. During this period, a rapid decrease in estriol levels is indicative of fetal distress.

This kit can be used in the risk assessment of Down's Syndrome (Trisomy 21) in combination with other biochemical and ultrasound parameters as specified above, taking also into account other data like maternal age and weight and using a validated software for Down's Syndrome risk assessment (***see Annex***):

1. **Triple test**: maternal serum AFP, hCG and unconjugated Estriol determination in the second trimester of pregnancy.
2. **Quadruple test**: maternal serum AFP, hCG, unconjugated Estriol and Inhibin-A determination in the second trimester of pregnancy.
3. **Integrated test**: maternal serum PAPP-A determination and nuchal translucency (NT) thickness measurement by an ultrasound scan in the first trimester of pregnancy and maternal serum AFP, hCG, unconjugated Estriol and Inhibin-A determination in the second trimester.

## Principle of method

This assay is based on the competition between unlabelled Estriol in standards and samples and a fixed quantity of $^{125}$I-labelled Estriol for a limited number of binding sites on Estriol-specific antibody. During incubation the antigen-antibody immuno-complex is immobilized on the reactive surface of test tubes, afterwards the reaction mixture is discarded and the radioactivity measured in a gamma counter. The concentration of antigen is inversely proportional to the radioactivity measured in test tubes.

By plotting binding values against a series of calibrators containing known amount of unconjugated Estriol, a calibration curve is constructed, from which the unknown concentration of unconjugated Estriol in patient samples can be determined.

## Contents of the kit

**1.** 1 bottle TRACER (55 ml), $^{125}$I-labelled Estriol in buffer with red dye and 0.1 % $NaN_3$, containing <220 kBq.

**2.** 1 bottle ANTISERUM (55 ml), in buffer with blue dye and 0.1 % $NaN_3$.

**3.** 6 vials STANDARD (6 x 0.5 ml), containing lyophilised human serum with 0.1% $NaN_3$. Exact standard concentrations are printed in the labels. *In the absence of an international reference preparation, standards have been prepared gravimetrically using highly pure (>99%) Estriol (SIGMA E1253).*

**4.** 1 vial CONTROL SERUM, lyophilised human serum with 0.1% $NaN_3$.

The concentration of the control serum is specified in the quality certificate enclosed.

**5.** 2 boxes of COATED TUBES, 2x50 pieces of 12x75 mm RIA tubes, packed in plastic boxes.

Quality certificate

Package insert

## Materials, tools and equipment required

Test tube rack allowing fixing of tubes, precision pipettes with disposable tips (50 and 500 µl), shaker, plastic foil, absorbent tissue, gamma counter

***Recommended tools and equipment***

Repeating pipettes

## 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 stored deep frozen (-20 °C). Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing cycles must be avoided. Hemolyzed and lipemic specimens may give false values and should not be used.

## Preparation of reagents, storage

Store all reagents of the kit between 2-8 °C before opening. At this temperature each reagent is stable until expiry date of the kit.

Add 500 µl distilled water to the lyophilised STANDARDS and CONTROL serum. Vortex gently (foaming should be avoided) and allow the solution to equilibrate at room temperature for at least 20 minutes. After reconstitution, store under **-20 °C** until expiry date for further use.

After opening, the TRACER and ANTISERUM solution can be stored between 2-8 °C until expiry 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.

## Assay procedure

*(For a quick guide , refer to Table 1.)*

1. Label coated tubes in duplicate for total counts (T), each standard (S1-S6), control serum (C) and samples (M).
2. Pipette 50 µl of standards, control and samples into the properly labelled tubes.
3. Pipette 500 µl of tracer into each tube.
4. Pipette 500 µl of antiserum into each tube except T. *This step shouldn't exceed 5 minutes between first and last tube, as a rule. Repeating pipettes recommended!*
5. Fix the test tube rack firmly onto the shaker plate. Seal all tubes with a plastic foil. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating in each tube (600-800 rpm recommended). Shake tubes for 2 hours at room temperature.
6. Decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
7. Count each tube for at least 60 seconds in a gamma counter. Calculate the unconjugated Estriol concentrations of samples as described below.

Table1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

| | T | S1-S6 | C | M |
|---|---|---|---|---|
| Standard | | **50** | | |
| Control | | | **50** | |
| Samples | | | | **50** |
| Tracer | **500** | **500** | **500** | **500** |
| Antiserum | | **500** | **500** | **500** |
| Shake for 2 hours at room temperature | | | | |
| Decant the fluid and blot on filter paper | | | | |
| Count radioactivity (60 sec/tube) | | | | |
| Calculate the results | | | | |

## Calculation of results

Calculate the average count per minute (cpm) for each pair of assay tubes.

Calculate (optionally) the percent $B_0/T$% for zero standard ($S_1$) by using the following equation:

$$B_0/T\% = \frac{S_1\ (cpm)}{T\ (cpm)} \times 100$$

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/B_0(\%) = \frac{S_{2\text{-}6} / C / M_x\ (cpm)}{S_1(cpm)} \times 100$$

For simplicity, these values are uncorrected for non-specific binding (NSB). This is enabled by low (less than 3 % of total) NSB.

Using semi-logarithmic graph paper plot $B/B_0$ (%) for each standard versus the corresponding concentration of unconjugated Estriol. Determine the unconjugated Estriol concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

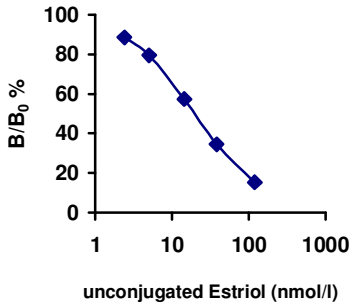



Figure 1. A typical standard curve
*(Do not use to calculate sample values!)*

Out of fitting programs applied for computerized data processing, logit-log or spline fittings can be used.

Table 2. Typical assay data

| Tubes | Mean cpm | B/T % | B/Bo% |
|---|---|---|---|
| T | 82536 | | |
| S1 | 55860 | 67.6 | |
| S2 | 49499 | 60.0 | 88.5 |
| S3 | 44445 | 53.8 | 79.5 |
| S4 | 32144 | 38.4 | 57.4 |
| S5 | 19310 | 23.4 | 34.6 |
| S6 | 8513 | 10.3 | 15.3 |
| C | 26647 | 32.3 | 47.7 |

## Characterization of assay

### Sensitivity

Analytical sensitivity is 0.4 nmol/l, as calculated by the interpolation of the mean plus two standard deviations of 14 replicates of the zero standard.

### Specificity

Cross reactivity values are shown below.

| Possible cross-reactants during pregnancy | Added nM steroid | Measured nM Estriol |
|---|---|---|
| Estriol-3-glucoronide | 100 | 3.0 |
| Estriol-3-sulfate | 1000 | 26.8 |
| 17ß-Estradiol | 1000 | 15.7 |
| Cortisol | 1600 | ND |
| Estrone | 7000 | 9 |
| Progesterone | 10000 | 2.2 |
| DHEA-$SO_4$ | 30000 | ND |

ND = non detectable

### Precision and reproducibility

7 control samples were assayed in 15 replicates to determine intra-assay precision. To determine inter-assay precision they were measured in duplicates in 10 independent assays. Values obtained are shown below.

| Intra-assay | | Inter-assay | |
|---|---|---|---|
| Mean nmol/l | CV% | Mean nmol/l | CV% |
| 3.9 | 6.4 | 3.5 | 6.3 |
| 9.3 | 3.9 | 8.7 | 5.9 |
| 12.0 | 2.6 | 11.3 | 2.1 |
| 21.4 | 3.9 | 20.6 | 5.4 |
| 55.2 | 3.6 | 51.7 | 4.1 |
| 80.7 | 4.1 | 76.8 | 3.8 |
| 97.6 | 4.2 | 89.5 | 3.8 |

### Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amounts of unconjugated Estriol. The average per cent recovery for 6 serum pools was101.7% (92% - 112%).

### Expected Values in the Second Trimeser of Pregnancy

Each laboratory should establish its own range of expected values. The values presented below should be used as a guideline only.

| Pregnancy Weeks | n | 1 MOM (nmol/l) |
|---|---|---|
| 14 | 22 | 6.4 |
| 15 | 1107 | 7.7 |
| 16 | 1019 | 9.3 |
| 17 | 137 | 11.2 |
| 18 | 62 | 12.9 |
| 19 | 22 | 14.9 |

The results obtained should only be interpreted in the context of the overall clinical picture. None of the *in vitro* diagnostic kits can be used as the one and only proof of any disease or disorder.

Every three months MoM values should be checked and, if necessary, recalculated.

**Conversion of SI units** can be performed according to the following formula:

1 nmol/l = 0.2884 ng/ml
1 ng/ml = 3.467 nmol/l

## Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient shaking, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. Uneven or incomplete shaking may result in a poor assay performance.

3) **Source of error!** Do not use a shaker in which some tubes can be exposed to heating. Do not place the shaker directly by an air conditioning or heating device or by an open window. Any differences in temperature between tubes during incubation can lead to serious measuring errors.

4) **Source of error!** If the incubation is not finished by decanting tubes but with aspiration of content, it is recommended to apply a washing step after it (with 2 ml physiological NaCl solution) in order to minimize errors due to unequal aspiration.

## Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

## Precaution

### Radioactivity

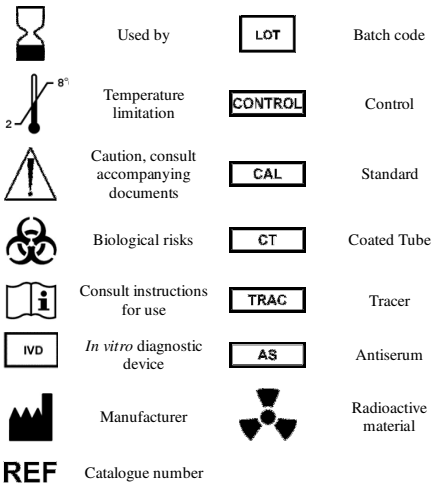
This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

### 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 immunoassay), 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 115 mg.




CE 1011

**WEB site: http://www.izotop.hu**
**Technical e-mail: immuno@izotop.hu**
**Commercial e-mail: commerce@izotop.hu**
IZOTOP
INSTITUTE OF ISOTOPES Ltd.
1535 Budapest. Pf.: 851.
Tel.: +36 1 392-2577, Fax: +36 1 395-9247

*Updated: 02.01.2015*

# Annex to the Instructions for Use
# In vitro kits for Down's Syndrome risk assessment

This kit has been validated for the risk assessment of 21 Trisomy, using the following kits and software:

| Kit / software name | Code | Manufacturer |
|---|---|---|
| PAPP-A IRMA | RK-4 CT | Institute of Isotopes Ltd. |
| Free βhCG IRMA | RK-820CT | Institute of Isotopes Ltd. |
| AFP IRMA | RK-800 CT | Institute of Isotopes Ltd. |
| hCG RIA | RK-770 CT | Institute of Isotopes Ltd. |
| Unconjugated Estriol RIA | RK-3 CT | Institute of Isotopes Ltd. |
| Active Inhibin-A ELISA | DSL-10-28100 | DSL - Beckman Coulter |
| αlpha - Antenatal Screening Software for Down's Syndrome and Neural Tube Defects. | | Logical Medical Systems Ltd. |
| *The kits and software listed before are CE marked. They can be used together for the risk assessment of Trisomy 21, according to the 98/79 EC IVD directive and based on the conformity assessment performed by an authorized notified body (**CE1011**).* | | |

Resumed results of validation, regarding efficiency of risk assessment:

**Sensitivity**

| Screening method | Number of tests performed | Positive cases with the test | | Cases confirmed by cytogenetic test | | Fals positive cases | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % of positive tests | No. | % |
| **Combined Test** | 1389 | 42 | 3,02 | 2 | 4,76 | 40 | 2,88 |
| **Quadruple Test** | 539 | 30 | 5,57 | 1 | 3,33 | 29 | 5,38 |
| **Integrated Test** | 1741 | 47 | 2,70 | 3 | 6,38 | 44 | 2,53 |

**Specificity**

| Screening method | Number of tests performed | Negative cases | Negative cases with the test | | Fals negative cases |
|---|---|---|---|---|---|
| | | | No. | % | No. (%) |
| **Combined Test** | 1389 | 1387 | 1345 | 96,97 | 1 (0,07) |
| **Quadruple Test** | 539 | 538 | 508 | 94,42 | 0 |
| **Integrated Test** | 1741 | 1738 | 1691 | 97,30 | 0 |

***Literature: see www.izotop.hu***

CE 1011

**WEB site: http://www.izotop.hu**
**Technical e-mail: immuno@izotop.hu**
**Commercial e-mail: commerce@izotop.hu**
IZOTOP
INSTITUTE OF ISOTOPES Ltd.
1535 Budapest. Pf.: 851.
Tel.: +36 1 392-2577, Fax: +36 1 395-9247