# Instructions for Use

# AFP ELISA RUO

RUO

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***Please use only the valid version of the Instructions for Use provided with the kit.***

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***For Research Use Only***

***Not for use in diagnostic procedures***

## 1 INTENDED USE

The **DRG AFP ELISA** is an enzyme immunoassay for the quantitative measurement of alpha fetoprotein (AFP) in serum.

**This kit is NOT intended to be used for the risk evaluation of trisomy 21**.

## 2 PRINCIPLE OF THE TEST

The DRG AFP ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the **sandwich principle**.

The microtiter wells are coated with a monoclonal (mouse) antibody directed towards a unique antigenic site on an AFP molecule. An aliquot of sample containing endogenous AFP is incubated in the coated well with enzyme conjugate, which is an anti-AFP antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase is proportional to the concentration of AFP in the sample.

Having added the substrate solution, the intensity of color developed is proportional to the concentration of AFP in the sample.

## 3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C - 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C - 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M $H_2SO_4$. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets.
    Safety Data Sheets for this product are available upon request directly from DRG.

## 4 REAGENTS

### 4.1 Reagents provided

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with anti-AFP antibody (monoclonal).
2. **Standard (Standard 0-4)**, 5 vials (lyophilized), 0.5 mL;
   Concentrations: 0 - 10 - 40 - 80 - 160 IU/mL
   Conversion: 1 IU/mL = 1.21 ng/mL
   The standards are calibrated against NIBSC 1st International Standard for Alphafoetoprotein AFP (AFP 1st IRP 72/225)
   See “Preparation of Reagents”;
   Contain non-mercury preservative.
3. ***Enzyme Conjugate***, 1 vial, 11 mL, ready to use,
   Anti-AFP antibody conjugated to horseradish peroxidase;
   Contain non-mercury preservative.
4. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
5. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.5M $H_2SO_4$,
   Avoid contact with the stop solution. It may cause skin irritations and burns.

**Note:** Additional *Standard 0* for sample dilution is available upon request.

### 4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm).
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled water
- Timer
- Graph paper or software for data reduction

### 4.3 Storage and stability of the Kit

When stored at 2 °C - 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C - 8 °C. Microtiter wells must be stored at 2 °C - 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for 9 weeks if stored as described above.

### 4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

***Standards***

Reconstitute the lyophilized contents of the standard vial with 0.5 mL distilled water and let stand for 10 minutes in minimum. Mix several times before use.

***Note:*** *The reconstituted standards are stable for 2 months at 2 °C - 8 °C.*
*For longer storage freeze at -20 °C.*

### 4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheets.

### 4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

## 5 SPECIMEN COLLECTION AND PREPARATION

Serum should be used in this assay.

*Note*: Samples containing sodium azide should not be used in the assay.

In general, it should be avoided to use hemolytic, icteric, or lipemic specimens. For further information refer to chapter “Interfering Substances”.

*NOTE: If an amniocentesis is necessary the specimen collection has to done before the puncture. After the amniotic puncture increased AFP values are determined.*

### 5.1 Specimen Collection

**Serum:**

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.

### 5.2 Specimen Storage

Specimens should be capped and may be stored for up to 7 days at 2 °C - 8 °C prior to assaying.

Specimens held for a longer time should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

### 5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:10: 10 µL sample + 90 µL *Standard 0* (mix thoroughly)

b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

## 6 TEST PROCEDURE

### 6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Optical density is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

### 6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **25 µL** of each ***Standard, control*** and **sample** with new disposable tips into appropriate wells.
3. Dispense **100 µL** ***Enzyme Conjugate*** into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **30 minutes** at room temperature.
5. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with distilled water (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   **Important note:**
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **100 µL** of ***Substrate Solution*** to each well.
7. Incubate for **10 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **50 µL** of ***Stop Solution*** to each well.
9. Measure the optical density of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)** with a microtiter plate reader.
   It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

### 6.3 Calculation of Results

1. Calculate the average optical density (OD) values for each set of standards, controls and samples.
2. Construct a standard curve by plotting the mean OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean OD value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4 Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

#### 6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

| Standard | Optical Density (450 nm) |
|---|---|
| Standard 0 (0 IU/mL) | 0.07 |
| Standard 1 (10 IU/mL) | 0.21 |
| Standard 2 (40 IU/mL) | 0.69 |
| Standard 3 (80 IU/mL) | 1.29 |
| Standard 4 (160 IU/mL) | 1.97 |

## 8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or DRG directly.

## 9 ASSAY CHARACTERISTICS

### 9.1 Assay Dynamic Range

The range of the assay is between 1.78 - 160 IU/mL.

### 9.2 Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

| Protein | added concentration | Cross-reactivity |
|---|---|---|
| HSA | 20 mg/mL | 0 % |
| Prolactin | 200 ng/mL | 3.0 % |
| hCG | 2000 ng/mL | 2.4 % |
| SP-1 | 2000 ng/mL | 0.3 % |
| HPL | 2,011 mg/mL | 3.2 % |

## 10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.

Any improper handling of samples or modification of this test might influence the results.

### 10.1 Interfering Substances

Hemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

### 10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of AFP in a sample.

### 10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 1600 IU/mL of AFP.

## 11 LEGAL ASPECTS

### 11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

### 11.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results are also invalid. In the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

**SYMBOLS USED**

| Symbol | English |
|---|---|
| RUO | For research use only |
| | Consult instructions for use |
| REF | Catalogue number |
| LOT | Batch code |
| | Contains sufficient for <n> tests |
| | Temperature limit |
| | Use-by date |
| | Manufacturer |
| | Caution |
| | |
| *Distributed by* | Distributed by |
| *Content* | Content |
| *Volume/No.* | Volume / No. |

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