DRG

コスモ・バイオ株式会社

DRG® PAPP-A ELISA (EIA-2397)

CE

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研究用

INTRODUCTION

The **DRG® PAPP-A Enzyme Immunoassay Kit** provides materials for the quantitative determination of Pregnancy associated plasma protein A (PAPP-A) in serum and plasma.

PAPP-A is a protein produced by the developing placenta. Its concentration in the maternal blood increases rapidly after the 7th week of pregnancy. The measurement of PAPP-A in the first trimester of pregnancy has been reported as a useful marker in antenatal screening for Down Syndrome and other fetal aneuploidies. Reduced PAPP-A values in combination with maternal age, the measurement of free ß-HCG and the ultrasonic determination of nuchal translucency (NT) in pregnancy weeks 11 to 14 may detect up to 90 % of pregnancies with Down syndrome (reference 7).

The DRG® PAPP-A ELISA EIA-2397 may be used for the risk assessment of Down syndrome (trisomy 21) in the first trimester of pregnancy. For the risk assessment of trisomy 21 and other fetal aneuploidies PAPP-A should always be measured in combination with other analytes (for example free ß-HCG and NT, see above) and a special software for the risk assessment of trisomy 21. According to the IVD Directive (98/79/EC) both software and kits for the additional analytes must be suitable for trisomy 21 screening and CE-certified by a notified body, indicated by the identification number of the notified body on the CE-mark on software and kits.

PRINCIPLE OF THE TEST

The DRG® PAPP-A ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a polyclonal anti-PAPP-A antibody. An aliquot of patient sample containing endogenous PAPP-A is incubated in the coated well with assay buffer. After incubation the unbound material is washed off. In the second incubation step a sandwich complex is formed with a polyclonal anti-PAPP-A antibody peroxidase conjugate. Having added the substrate solution, the intensity of color developed is proportional to the concentration of PAPP-A in the patient sample.

PRECAUTIONS

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- 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.
- Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and Microtiterplate Readers.
- 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.

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- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request directly from DRG International, Inc.
- The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

KIT COMPONENTS

Contents of the Kit

- **Microtiterwells**, 12x8 (break apart) strips, 96 wells
  Wells coated with polyclonal anti-PAPP-A antibody
- **Standard** (Standard 0-5), 6 vials (lyoph.), 0.15 ml
  0; 1; 2.5; 5.0; 15.0; 30.0 µg/ml
  Conversion: 1 mU/ml = 4.5 mg/l
  The DRG® PAPP-A Standards are comparable with NEQAS approved Reference material for Down Syndrome Screening (U/L, IRP 76/610)
  see "Preparation of Reagents"
- Standards contain < 0.3% Proclin as a preservative.
- **Enzyme Conjugate**, 1 vial, 1.5 ml, 10X conc.
  Complex containing horseradish peroxidase
  see "Preparation of Reagents"
- Enzyme conjugate contains < 0.3% Proclin as a preservative.
- **Conjugate Diluent**, 1 vial, 14 ml
- **Substrate Solution**, 1 vial, 14 ml, ready to use TMB
- **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.
- **Assay Buffer**, 1 vial, 25 ml, ready to use
- **Control (low and high)**, 2 vials, (lyoph.), 0.15 ml
  (exact control ranges see vial label)
  see "Preparation of Reagents"
- Controls contain < 0.3% Proclin as a preservative.
- **Wash Solution**, 1 vial, 30 ml (40X concentrated)
  see "Preparation of Reagents"

Equipment and material required but not provided

– A microtiterplate calibrated reader (450±10 nm)(e.g. the DRG International Microtiterplate Reader).
– Calibrated variable precision micropipettes.
– Absorbent paper.
– Aqua dest.

Storage and stability of the Kit

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

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

Preparation of Reagents

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

Standards

Reconstitute the lyophilized contents of the standard vial with 150 µl Aqua dest.

Controls

Reconstitute the lyophilized controls with 150 µl Aqua dest. each.

Wash Solution

Dilute 30 ml of concentrated Wash Solution with 1170 ml deionized water to a final volume of 1200 ml.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Enzyme Conjugate

30 min. before use dilute 1.0 ml of concentrated Enzyme Conjugate with 10 ml Conjugate Diluent.

Note: *The Enzyme Conjugate has to be **prepared fresh 30 min. before use** and cannot be stored longer than 24 hours. If more than one test run is performed, dilute only the quantity required for each test run.*

Disposal of the Kit

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

Damaged Test Kits

In case of any severe damage of the test kit or components, DRG® has to be informed in writing, no later than 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.

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SPECIMEN

Serum or plasma (EDTA-, Heparin- or citrat plasma) can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.

Specimen Collection

Serum:
Collect blood by venipuncture (e.g Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.
Plasma:
Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.
(E.g for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001; for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001; for Citrat plasma Sarstedt Monovette – green cap - # 02.167.001.)

Specimen Storage

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying.
If EDTA-plasma is stored at 2-8 °C, it must be assayed within 48 hours. Specimens held for a longer time (up to two months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

Samples with expected values greater than 30 µg/ml should be diluted with *Standard 0* before assaying.
For the calculation of the concentrations this dilution factor has to be taken into account
Example: dilution 1:10: 10 µl Serum + 90 µl Standard 0 (mix thoroughly)

TEST PROCEDURE

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 pipet tips for each standard, control or sample in order to avoid cross-contamination
- Absorbance 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.

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Assay Procedure

All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same. Each run must include a standard curve.

1. Secure the desired number of Microtiterwells in the holder.
2. Dispense **10 µl** of each Standard, Controls and samples with new disposable tips into appropriate wells.
3. Add **100 µl** Assay Buffer into each well
4. Incubate for **30 minutes** at room temperature.
5. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (400 µl). Strike the wells sharply on absorbent paper to remove residual water droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Dispense **100 µl** diluted Enzyme Conjugate (see "Preparation of Reagents") into each well.
7. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
8. Incubate for **30 minutes** at room temperature.
9. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution. Strike the wells sharply on absorbent paper to remove residual water droplets.
10. Add **100 µl** of Substrate Solution to each well.
11. Incubate for **15 minutes** at room temperature.
12. Stop the enzymatic reaction by adding **50 µl** of Stop Solution to each well.
13. Read the OD at **450±10 nm** with a microtiterplate reader **within 10 minutes** after adding the Stop Solution.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 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.

Below is listed a typical example of a standard curve with the PAPP-A ELISA.

Standard	**Optical Units (450 nm)**
Standard 0 (0 µg/ml)	0.18
Standard 1 (1 µg/ml)	0.38
Standard 2 (2.5 µg/ml)	0.56
Standard 3 (5 µg/ml)	0.83
Standard 4 (15 µg/ml)	1.44
Standard 5 (30 µg/ml)	1.80

EXPECTED VALUES
It is strongly recommended that each laboratory should determine its own normal and abnormal values.

Pregnant women in the 1st trimester
238 samples of pregnant women in the 1st trimester have been measured with the DRG® PAPP-A ELISA. The values are validated in comparison with a Gaussian distribution.

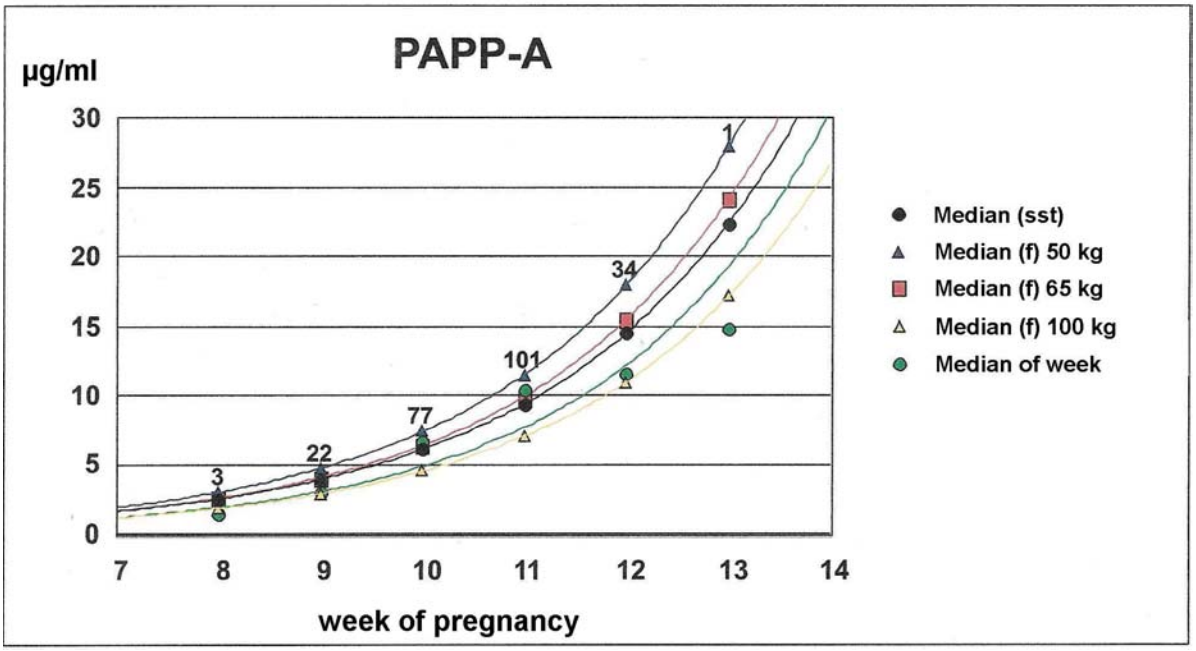
Consideration of body weight and day of gestation results in the following regression equation:

Median (f) PAPP-A = EXP (-2.12268 + 0.06324 * gestation day – 0.00979 * body weight).

If the values of the same 238 pregnant women are compared with the gestation day only (body weight not considered) the following weight independent regression equation is found:

Median (sst) PAPP-A = EXP (-2.705444 + 0.0618725 * gestation day).

In the following diagram and table the medians of function (median (f)) for **completed pregnancy weeks** 8 to 13 have been calculated for three body weights (50 kg, 65 kg (mean body weight), and 100 kg). For comparison the medians were also determined manually (Median of week) and by using the weight independent regression equation (Median (sst)).



Completed week of gestation	day of gestation	Median(sst) [µg/ml] weight independent	Median (f) [µg/ml] weight 50 kg	Median (f) [µg/ml] weight 65 kg	Median (f) [µg/ml] weight 100 kg	Median of week [µg/ml]
8	59	2.57	3.06	2.6	1.88	1.5
9	66	3.97	4.77	4.1	2.92	3.0
10	73	6.12	7.42	6.4	4.55	6.7
11	80	9.43	11.55	10.0	7.08	10.5
12	87	14.55	17.99	15.5	11.03	11.6
13	94	22.43	28.00	24.2	17.17	14.9

Population and laboratory differences may lead to slightly different medians. Each laboratory should therefore determine and continuously update its own medians from its own patient collective. The regression equations and values in the table should be used as a guideline only. The calculation of medians and/or regression functions for the calculation of medians from own patient databases should be performed with the applied trisomy 21 risk calculation software. Medians determined for the DRG® PAPP-A ELISA cannot be used with assays of other manufacturers. Medians determined for PAPP-A assays of other manufacturers cannot be used with the DRG® PAPP-A ELISA.

1.1.1 Use for Down Syndrome Screening

For risk calculation in prenatal screening PAPP-A concentrations are indicated as MOM (multiple of medians, MOM = Measured Concentration (PAPP-A) / Median PAPP-A).
In Down syndrome pregnancies, the median of MOMs for PAPP-A are increasing during the first trimester and are not distinguishable anymore from normal pregnancies during the second trimester (reference 6, details see table). PAPP-A must therefore be measured in the first trimester of pregnancy (completed weeks 10 – 13).

Completed week of pregnancy	10	11	12	13	14-20
Median of MOM in pregnancies with Down Syndrome	0,34	0,42	0,50	0,58	1,11

Data from reference 6

For risk calculation of trisomy 21 not only PAPP-A but also other parameters like free ßHCG and nuchal translucency (NT) for the 1st trimester and/or AFP, free Estriol and HCG for the 2nd trimester have to be determined.
The use of these parameters for risk calculation of trisomy 21 requires a special software. **According to the IVD Directive (98/79/EC) both software and kits for the additional analytes must be suitable for trisomy 21 screening and CE-certified by a notified body, indicated by the identification number of the notified body on the CE-mark on software and kits. The software must allow the calculation of medians from own patient measurements.**

It is imperative to take into consideration additional factors, e.g. age of the woman, weight, ethnic group and smoker/non-smoker. **An underestimation of the gestation age can lead to a falsely calculated high risk (false positive).** To reduce this source of error, it is important to **determine the gestation age as precisely as possible. Gestation age calculation from the last menstrual cycle has an inherently high risk of variation. Sonographic determination of the crown-rump length (CRL) or biparietal diameter (BIP)** is recommended for the proper determination of the gestation age.
PAPP-A measurement in the course of a prenatal screening determines only a risk for trisomy 21.
For proof of trisomy 21 genetic determinations are required.

ASSAY CHARACTERISTICS

Assay Dynamic Range

The range of the assay is between 0 – 30 µg/ml.

Specificity of Antibodies (Cross Reactivity)

The antibody used for the DRG® PAPP-A ELISA is specific for human PAPP-A. There is no cross-reactivity to other species.
No reaction is seen with normal human plasma.

Analytical Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of *Standard 0* and was found to be < 0.133 µg/ml for a serum sample.

Accuracy

Quality Control

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. Use controls at both normal and pathological levels.
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 analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient 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.

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Recovery

Sample	Added Conc. (µg/ml)	Measured Conc. (µg/ml)	Expected Conc. (µg/ml)	Recovery (%)
1	--	19,89	19,89	100
	1,25	10,94	11,20	97,7
	2,50	12,00	12,45	96,4
	7,50	17,66	17,45	101,2
	15,00	24,78	24,95	99,3
2	--	2,17	19,89	100
	1,25	2,44	2,34	104,3
	2,50	3,44	3,59	96,0
	7,50	9,00	8,59	104,8
	15,00	15,77	16,09	98,1

Linearity

Sample	Dilution	Mean Conc. (µg/ml)	Recovery (%)
1	None	20,90	100
	1:2	10,30	98,5
	1:4	5,39	103,1
	1:8	2,61	100,0
	1:16	1,25	95,8
2	None	11,83	--
	1:2	5,80	98,1
	1:4	2,82	95,3
	1:8	1,45	98,1
	1:16	0,73	98,8

Precision

Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean (µg/ml)	CV (%)
1	20	1.12	2.89
2	20	10.17	2.81

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Inter Assay Variation

The between assay variability is shown below:

Sample	n	Mean (µg/ml)	CV (%)
1	12	1.18	7.18
2	12	10.94	5.72

LIMITATIONS OF USE

Interfering Substances

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

Drug Interferences

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

High-Dose-Hook Effect

No hook effect was observed in this test up to 300 µg/ml of PAPP-A.

LEGAL ASPECTS

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®.

Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 10.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutical consequences.

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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 subject to point 10.2. are also invalid. Regardless, 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.

REFERENCES

1. Spencer K., Scouter V., Tul N., Snijders R., Nicolaides K.H. Ultrasound Obset Gynecol. 1999; 13:1-7
2. Sancken U. Deutsches Ärzteblatt 97 3.März 2000, Heft 9:A532-A537
3. Bayes-Genis A., Conover C.A., Overgaard M.T., et al.
   N. Engl. J. Med., Vol.345, No.14, 2001: 1022-1029
4. Schaeffer H.-J., Sancken U., Eiben B., Opper C., Jung A. Fetal Diagn Ther. 2001; 16: 437-464
5. Qiu-Ping Q., Christiansen M., Oxvig C., Pettersson K., Sottrup-Jensen L., Koch C.,
   Norgaard-Pedersen B. Clinical Chemistry 43:12, 1997; 2323-2332
6. Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. Health Technol Assess. 2003; 7(11). First and second trimester antenatal screening for Down´s Syndrom: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS).
7. Bindra R, Heath V, Liao A, Spencer K, Nicolaides KH. Ultrasound Obstet Gynecol. 2002 Sep;20(3):219-25. One-stop clinic for assessment of risk for trisomy 21 at 11-14 weeks: a prospective study of 15 030 pregnancies.