DRG® Amyloid beta Auto-Ab (serum) (EIA-5099)

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As of 14 Dec. 2010 rm (Vers. 1.1)

1 INTRODUCTION

Intended Use

The **DRG Amyloid β Auto-Ab ELISA** is an enzyme immunoassay for measurement of autoantibodies against Amyloid Beta (Aβ) in human serum

For research use only. Not for use in diagnostic procedures.

2 PRINCIPLE OF THE TEST

The DRG Amyloid β Auto-Ab ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with (1-42) β-Amyloid. An aliquot of sample containing Aβ-autoantibodies is incubated in the coated well with assay buffer. After washing a second incubation with enzyme conjugate, which is goat anti-human IgG conjugated to horseradish peroxidase, follows. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase is proportional to the concentration of autoantibodies against Aβ in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of autoantibodies against Aβ in the sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for research 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 the package insert 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 to 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 (21-26°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.

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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 Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DRG.

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4 REAGENTS

4.1 Reagents provided

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with (1-42) β-Amyloid.
2. ***Standard (Standard 1-6)***, 6 vials (lyophilized), 0.1 mL;
   Concentrations: 1.0 – 5.0 – 15.0 – 30.0 – 60.0 – 120 RTU
   see „Reagent Preparation“
   contain mercury free preservative.
3. ***Control***, 1 vial (lyophilized), 0.1 mL,
   see „Reagent Preparation“
   For control values and ranges please refer to vial label or QC-Datasheet.
   contains mercury free preservative.
4. ***Sample Diluent***, 1 vial, 25 mL, ready to use,
   contains mercury free preservative.
5. ***Assay Buffer***, 1 vial, 25 mL, ready to use
   contains mercury free preservative.
6. ***Enzyme Conjugate***, 1 vial, 20 mL, ready to use,
   goat anti human IgG conjugated to horseradish peroxidase;
   contains mercury free preservative.
7. ***Substrate Solution***, 1 vial, 25 mL, ready to use,
   Tetramethylbenzidine (TMB).
8. ***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.
9. ***Wash Solution***, 1 vial, 30 mL (40X concentrated),
   see „Preparation of Reagents“.

Note: Additional *Sample Diluent* for sample dilution is available upon request.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm) (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C to 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 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kit components (with exception of reconstituted standard) retain activity for two months if stored as described above.

4.4 Reagent Preparation

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

Standards and Control

Reconstitute the lyophilized contents of each vial with 100 µL *Sample Diluent.*

Note: *The reconstituted standards and control are stable for one day at 2 °C to 8 °C.*

The reconstituted standards and control have to be diluted **1 + 10** with *Sample Diluent*;
e.g. 10 µL *Standard* or *Control* + 100 µL *Sample Diluent*

This prediluted standards and control have to be used immediately. Remaining volumes have to be discarded.

Wash Solution

Add deionized water to the 40X concentrated 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.

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 Material Safety Data Sheet.

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 can be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

5.1 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. Samples containing anticoagulant may require increased clotting time.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying.

Specimens held for a longer time (up to 12 months) 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

Prior to assay the samples have to be diluted **1 + 10** with Sample Diluent;
e.g. 10 µL sample + 100 µL *Sample Diluent* (mix thoroughly)

Prediluted samples have to be used immediately. Remaining volumes have to be discarded.

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

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

Example:

dilution 1:10: 10 µL diluted sample + 90 µL *Sample Diluent* (mix thoroughly)

6 ASSAY 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.
- 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.

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 **20 µL** of each prediluted ***Standard, Control*** and prediluted **samples** with new disposable tips into appropriate wells. (See" Reagent Preparation" and "Specimen Dilution")
3. Dispense **200 µL** ***Assay Buffer*** into each well.
4. Incubate for **30 minutes** on a shaker (300-500 rpm) at room temperature.
5. Briskly shake out the contents of the wells.
   Rinse the wells **4 times** with diluted ***Wash Solution*** (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. Dispense **150 µL** ***Enzyme Conjugate*** into each well.
7. Incubate for **30 minutes** on a shaker (300-500 rpm) at room temperature.
8. Briskly shake out the contents of the wells.
   Rinse the wells **4 times** with diluted ***Wash Solution*** (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
9. Add **150 µL** of ***Substrate Solution*** to each well.
10. Incubate for **10 minutes** at room temperature.
11. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
12. Determine the absorbance (OD) of each well at **450 ± 10 nm** 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 absorbance values for each set of standards, controls and samples.
2. Using semi-logarithmic graph paper, 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. 4 Parameter Logistics is the preferred method. 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 or reported as > 120 RTU. 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 Units (450 nm)
Standard 1 (1 RTU)	0.22
Standard 2 (5 RTU)	0.27
Standard 3 (15 RTU)	0.41
Standard 4 (30 RTU)	0.63
Standard 5 (60 RTU)	1.2
Standard 6 (120 RTU)	2.5

7 REFERENCES / LITERATURE

1. Sambamurti, K., et al., Neuromolecular Med., 1, 1-31 (2002).
2. Lorenzo, A., et al., Nat. Neurosci., 3, 460-464 (2000).