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User's Manual

Serotonin ELISA Kit

REF **DEIA2014**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Ultra-sensitive Enzyme Immunoassay for the quantitative determination of Serotonin. Flexible test system for various biological sample types and volumes.

Principles of Testing

Serotonin is acylated and detected by the subsequent competitive ELISA kit, which uses the microtiter plate format. Antigens are bound to the solid phase of the microtiter plate. The acylated standards, controls and samples and the solid phase bound analytes compete for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm. Quantification of unknown samples is achieved by comparing their absorbance with a standard curve prepared with known standard concentrations.

Reagents And Materials Provided

1. Standards and Controls (Concentrated)

Component	Colour/Cap	Concentration after dilution				Volume/ Vial
		ng/ml	nmol/l	pg/sample vol.	pmol/sample vol.	
STANDARD A	white	0	0	0	0	4 ml
STANDARD B	light yellow	0.015	0.085	1.5	8.5	4 ml
STANDARD C	orange	0.05	0.28	5	28.4	4 ml
STANDARD D	dark blue	0.15	0.85	15	85	4 ml
STANDARD E	light grey	0.5	2.8	50	284	4 ml
STANDARD F	black	2.5	14	250	1 418	4 ml
CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!				4 ml
CONTROL 2	dark red					4 ml

Conversion: Serotonin (ng/ml) × 5.67 = Serotonin (nmol/l), Serotonin (pg/sample volume) × 5.67 = Serotonin (pmol/sample volume)

Content: TRIS buffer with non-mercury preservative, spiked with defined quantity of serotonin.

Note:Standards and controls have to be diluted 1+1000 prior to use. The shown concentrations apply to diluted standards and controls (1+1000) when 100 µl of undiluted sample is used.

2. Acylation Plate: 1 × 96 well plate, pre-coated with acylation reagent. Ready to use.
3. Stabilizer: Stabilizing agent, 10% solution, 1 × 4 ml/vial, purple cap. Ready to use.
4. Dilution Concentrate (20×): TRIS buffer with 1% stabilizing agent and a non-mercury preservative, 1 × 50 ml/vial, white cap.
5. Adhesive Foil: Adhesive Foils in a resealable pouch, 1 × 4 foils. Ready to use.
6. Wash Buffer Concentrate (50×): Buffer with a non-ionic detergent and physiological pH, 1 × 20 ml/vial, light purple cap.
7. Enzyme Conjugate: Goat anti-rabbit immunoglobulins conjugated with peroxidase, 1 × 12 ml/vial, red cap. Ready to use.

8. Substrate: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide, 1 × 12 ml/vial, black cap. Ready to use
9. Stop Solution: 0.25 M sulfuric acid, 1 × 12 ml/vial, light grey cap. Ready to use.
10. Serotonin Antiserum: Rabbit anti-serotonin antibody, blue coloured, 1 × 3 ml/vial, blue cap. Ready to use.
11. Acylation Buffer: TRIS buffer with non-mercury preservative, 1 × 4 ml/vial, yellow cap. Ready to use.
12. Serotonin Microtiter Strips: 1 × 96 well (12×8) antigen precoated microwell plate in a resealable pouch with desiccant. Ready to use.

Materials Required But Not Supplied

1. Calibrated precision pipettes to dispense volumes between 1 – 100 µl; 10 ml
2. Microtiter plate washing device (manual, semi-automated or automated)
3. ELISA reader capable of reading absorbance at 450 nm and if possible 620 – 650 nm
4. Plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
5. Absorbent material (paper towel)
6. Water (deionized, distilled, or ultra-pure)
7. Vortex mixer

Storage

Store the unopened reagents at 2-8°C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2-8°C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

Specimen Collection And Preparation

Specimen collection and storage

In general this assay is dedicated for any biological sample such as serum, urine, platelets, platelet-poor plasma, tissue homogenates, dialysates and other samples.

Storage: up to 6 hours at 2-8 °C, for longer periods (up to 6 months) at -20 °C. Avoid exposure to direct sunlight.

1. Serum: Collect blood by venipuncture (Monovette™ or Vacuette™ for serum), allow to clot, and separate serum by centrifugation according to manufacturer's instructions at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time. Haemolytic and especially lipemic samples should not be used for the assay.
2. Urine: Spontaneous or 24-hour urine, collected in a bottle containing 10 – 15 ml of 6 M HCl, should be used. Determine the total volume of urine excreted during a period of 24 h for calculation of the results.
3. Platelets: More than 98 percent of the circulating serotonin is located in the platelets and is released during blood clotting. Blood must be collected according to manufacturer's instructions by venipuncture in plastic tubes (Monovette™ or Vacuette™) containing EDTA or Citrate as anticoagulant. To obtain platelet-rich plasma (PRP) the samples are centrifuged for 10 minutes at room temperature (200 × g). Transfer the supernatant to another tube and count the platelets. The platelet pellet is obtained by adding 800 µl of

physiological saline to 200 µl of PRP (containing between 350,000 – 500,000 platelets/µl) and centrifugation (4,500 × g, 10 minutes at 4 °C). The supernatant is then discarded. 200 µl of water (deionized, distilled, or ultra-pure) is added to the pellet and mixed thoroughly on a vortex mixer. This suspension can be stored frozen for several weeks at < -20°C. After thawing of the frozen samples, centrifuge at 10,000 × g for 2 minutes at room temperature.

Sample preparation

The Serotonin Research ELISA is a flexible high sensitive test system for various biological sample types and sizes. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to fit the protocol to his specific needs.

1. Using poorly diluted or undiluted samples might lead to incorrect results due to a matrix effect. Therefore it is advisable to perform a linearity experiment prior to the test. Make different dilutions of a sample with the included diluent (e.g. 1:1, 1:4, 1:10, 1:20 and so on), spike each dilution with the same known concentration and check the recovery. If the samples are found consistently correct and no matrix effect is detected, samples can be used undiluted.
2. If a matrix effect is detected, samples should be diluted with the included diluent prior to the test. It is also possible to dilute the standards with the sample matrix instead of diluent, in order to create the same matrix conditions for standards and samples. Currently the following buffers/solvents are evaluated for use: Ringer Buffer, PBS and 0.9% NaCl. If another substance is used, please check the compatibility by a Proof of Principle prior to the measurement. Prepare a stock solution of serotonin or use standard F. Add small amounts (to change the native sample matrix as little as possible) of the stock solution or standard F to the sample matrix and check the recovery. Please take the correction factor into account.
3. If the expected sample concentrations are higher than the standard range samples should be diluted accordingly with the included diluent. Please take the correction factor into account.
4. The measuring range and sensitivity of this test are defined by the correction factor, which is calculated by sample volume and dilution. If the expected concentrations are unknown, please test different dilutions and amounts of sample volume, to make sure that the samples will fall into the measuring range of this assay.
5. Serotonin decomposes fast in acidic solution (< pH 3) and at higher temperatures.
6. When acidic sample solutions are used, protect serotonin by keeping the temperature low (2 -8 °C). Use pre-cooled buffers and materials. Adjust the pH to (6 – 7.4) as soon as possible.
7. A pH 7 - 8.5 during acylation is mandatory.
8. To protect serotonin against oxidative degradation add Stabilizer. If you need any support in establishing a protocol for your specific purposes, do not hesitate to contact the manufacturer directly!

Reagent Preparation

1. Diluent: Dilute the 50 ml Dilution Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml. The Diluent (Diluted Dilution Concentrate) contains 1% Stabilizer.

   Storage: 1 month at 2 – 8 °C
2. Wash Buffer: Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.

   Storage: 1 month at 2 – 8 °C
3. Standards and controls: The standards and controls have to be diluted freshly 1 + 1000 with Diluent or buffer*[1] , for example: 10 µl standard + 10 ml Diluent or buffer*[1] .

* 1 The buffer used for the respective experiment, enriched with 1% Stabilizer.

4. Serotonin Microtiter Strips: In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

Assay Procedure

Acylation

100 µl of the acylated standards, controls and samples are needed for the subsequent ELISA.

1. Pipette 100 µl of diluted standards, diluted controls, and 1 – 100 µl of samples into the respective wells of the Acylation Plate*2.
2. Add Diluent or buffer to the wells containing the samples to a final volume of 100 µl.
3. Add 25 µl Acylation Buffer to all tubes.
4. Acylate for 30 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).

*2 The wells of the Acylation Plate are covered by plastic bars which have to be removed prior to use.

Serotonin ELISA

1. Pipette 100 µl of the acylated standards, controls and samples into the appropriate wells of the Serotonin/5-HIAA Microtiter Strips.
2. Pipette 25 µl of the Serotonin Antiserum into all wells.
3. Cover plate with Adhesive Foil and incubate for 15 - 20 h at 2 – 8 °C.
4. Remove the foil. Discard or aspirate the content of the wells. Wash the plate 3× by adding 300 µl of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
5. Pipette 100 µl of the Enzyme Conjugate into all wells.
6. Incubate for 30 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
7. Discard or aspirate the content of the wells. Wash the plate 3× by adding 300 µl of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
8. Pipette 100 µl of the Substrate into all wells and incubate for 20 - 30 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm). Avoid exposure to direct sunlight!
9. Add 100 µl of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm (if possible, a reference wavelength between 620 nm and 650 nm is recommended).

Quality Control

The confidence limits of the kit controls are listed in the QC-Report.

Calculation

The calibration curve from which the concentrations of the samples can be read off, is obtained by plotting the absorbance readings (calculate the mean absorbance) measured for the standards (linear, y-axis) against

the corresponding standard concentrations (logarithmic, x-axis). The use of a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima) is recommended.

This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.

The concentrations of the samples taken from the standard curve have to be multiplied by a correction factor:

Correction factor = 100 µl (volume of standards) / sample volume (µl)

Three different examples are shown to illustrate the calculation of possible correction factors:

Example 1)

10 µl of the undiluted sample are acylated and the concentration taken from the standard curve is 0.02 ng/ml serotonin.

Correction factor = 100/10 = 10

Final concentration of the sample = 0.02 ng/ml × 10 = 0.2 ng/ml serotonin

Example 2)

100 µl of the 1:100 prediluted sample are acylated and the concentration taken from the standard curve is 0.02 ng/ml serotonin.

Correction factor = 100

Final concentration of the sample = 0.02 ng/ml × 100 = 2.0 ng/ml serotonin

Example 3)

10 µl of the 1:100 prediluted sample are acylated and the concentration taken from the standard curve is 0.02 ng/ml serotonin.

Correction factor = 100 × (100/10) = 1000

Final concentration of the sample = 0.02 ng/ml × 1000 = 20 ng/ml serotonin

Conversion: Serotonin (pg/ml) × 5.67 = Serotonin (pmol/l)

Serotonin (pg/sample volume) × 5.67 = Serotonin (pmol/sample volume)

Sensitivity

0.005 ng/ml × correction factor

Specificity

img align="absmiddle" alt="" style="max-width: 400px" border="0" src="https://img2.creative-diagnostics.com/productimages/DEIA2014-SPECIFICITY.jpg">

Precautions

1. This kit is intended for professional use only. Users should have a thorough understanding of this protocol

for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.

2. The principles of Good Laboratory Practice (GLP) have to be followed.
3. In order to reduce exposure to potentially harmful substances, wear lab coats, disposable latex gloves and protective glasses where necessary.
4. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
5. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
6. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
7. Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
8. Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
9. Incubation times do influence the results. All wells should be handled in the same order and time intervals.
10. To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
11. A standard curve must be established for each run.
12. The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
13. Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
14. Avoid contact with Stop Solution containing 0.25 M H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
15. 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.
16. For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
17. Kit reagents must be regarded as hazardous waste and disposed according to national regulations.