For in-vitro use only! Instructions for use **Omega Diagnostics GmbH**

ECP

CE REF 36061000

Enzyme immunoassay for the quantitative determination of human Eosinophilic Cationic Protein in serum. Micro-titration plate version for 96 tests

1. Intended use

Enzyme immunoassay for the quantitative determination of human Eosinophilic Cationic Protein in serum. Determination of specific ECP with this test kit is validated in association with the Omega Diagnostics test system and the determined performance data have been established for the Omega Diagnostics test systems. For the use with other test systems the validation has to be performed by the user. Use is restricted to qualified specialists, who have been specially instructed and trained in processes which are carried out with the use of IVDs.

2. Introduction

Eosinophilic Cationic Protein (ECP), Eosinophilic Derived Neurotoxin (EDN) and Major Basic Protein (MBP) are the best-known protein mediators from the activated eosinophils. ECP and EDN occur in the granule matrix of eosinophilic granulocytes, MBP occurs in the granule nucleus. ECP and EDN belong to the ribonuclease A family. ECP and MBP are characterised by a high level of cyto-toxicity. Activated eosinophilic granulocytes play a major role in asthmatic disorders. As ECP is released from eosinophilic granulocytes, it can be used as a marker for eosinophil activation and degranulation. The ECP test kit is a highly sensitive test system for the measurement of the release of ECP.

3. Test Principle

The ECP test kit is a sandwich-ELISA and detects human ECP with a detection limit of approximately 0.4 ng/ml. No cross-reaction with EDN has been observed. The solid phase is a micro-titration plate well, to which an anti-human ECP antibody (monoclonal) is bound. In the first step, the patient's serum or standard serum is pipetted into the well of the micro-titration plate. The ECP becomes bound to the anti-human ECP which is bound to the solid phase. After a washing stage, an anti-human ECP antibody (polyclonal) labeled with horseradish peroxidase is pipetted into the well of the micro-titration plate. An anti-human ECP/anti-human ECP (enzyme) complex is produced. After a further washing stage, the substrate solution is pipetted into the well of the micro-titration plate. Due to the enzymatic activity, a coloured solution is produced from the substrate. Then the enzymatic reaction is terminated by the addition of a stop solution. The quantity of the bound, labeled anti-human ECP antibody is proportional to the quantity of the released ECP. The measurement is performed in a photometer at 450 nm. A standard curve is produced from the measured standard serums. Calculation of the patient's values is made on the basis of the standard curve.

4. Contents of the ECP test kit

1. CONJ Conjugate: 1 bottle with 15 ml polyclonal anti-human ECP antibodies, conjugated with horseradish peroxidase in a buffered protein solution. Contains 1% BSA.
2. WASH | 20x Washing solution (concentrate): 1 bottle with 100 ml concentrated washing solution with T-PBS (for preparation of the washing solution see 10.6).
3. SUBS Substrate: 1 bottle with 20 ml tetramethyl-benzidine/H_2O_2.
4. STOP Stop solution: 1 bottle with 20 ml 0.5 M sulphuric acid.
5. DIL | AS Diluent solution: 1 bottle with 50 ml diluent solution; contains 1% goat serum. Preservation agent: 0.09 % sodium azide.
6. CAL | SERUM Calibration system: 5 bottles, each with 1.3 ml serum with various concentrations of ECP. Contains 1% goat serum. Preservation agent: 0.09 % sodium azide.
   The calibrators are filled in increasing concentrations:
   - CAL | SERUM | 1 Calibrator 1 = 0.4 ng/ml;
   - CAL | SERUM | 2 Calibrator 2 = 1.2 ng/ml;
   - CAL | SERUM | 3 Calibrator 3 = 4.0 ng/ml;
   - CAL | SERUM | 4 Calibrator 4 = 12 ng/ml;
   - CAL | SERUM | 5 Calibrator 5 = 40 ng/ml.
7. MTP Micro-titration strips: 12 micro-titration strips (individually removable) each with 8 wells, coated with anti-human ECP antibodies.

5. Additionally materials and devices

Materials and equipment:

- Micro-pipette with disposable tips, 50 µl and 100 µl
- Manual hand dispenser e.g. Eppendorf Multipete with Combitips 5 ml
- Measuring cylinders, 100 and 1,000 ml
- Adhesive film or micro-titration plate cover
- Micro-titration plates (non-coated, flat base) Greiner
- Disposable gloves
- 8-channel pipette (100 µl) with disposable tips
- Distilled water
- Stop watch
- Printer
- Micro-titration plate photometer 450 nm (e.g. TECAN Spectra or TECAN Sunrise)
- Washer for micro-titration plates (e.g.TECAN-Columbus or Hydroflex)
- Vacutainer SST blood sample vials (Bection Dickinson) or S-Monovette with serum gel (Sarstedt)
- Laboratory centrifuge
- Omega Diagnostics Alpha-/Quattro-System

6. Limitations of the procedure

- Reliable and reproducible results can only be obtained if the test is performed correctly (see test procedure, Section 10).
- An ECP calibration system must be measured for each micro-titration plate.
- Evidence of increased ECP values only demonstrates an increased activation of eosinophils. The clinical diagnosis must include further data for a possible diagnosis.
- Positive results for total ECP in-vitro test need not automatically cause clinical symptoms.
- Incorrectly increased or reduced ECP values may occur if:
  - incorrect blood sample vials are used;
  - the coagulation time is not precisely adhered to;
  - the coagulation temperature is not precisely adhered to;
  - the transfer of serum into a new vial is not carried out, or is not carried out in time.
- The release of ECP can be impaired by corticosteroid treatment (see Reference 5) or by cytostatics and immunosuppressive drugs.

7. Specific performance data

- Analytical sensitivity
  The average dependence of the measurement signal from the sample concentration in the clinically relevant range (see Section 12) can be described as follows:
  y = 0.030 x
- Analytical specificity
  A cross-reaction with EDN (Eosinophil Derived Neurotoxin), a functionally related protein, has not been observed up to a concentration of 500 ng/ml.
- Accuracy 73 - 100 %*
- Repeatability (Intra-assay) < 12 CV %*
- Reproducibility (Inter-assay) < 11 CV %*
- Average reproducibility (Inter-batch) 18.7 CV %*
- Lowest detection level < 0.4 ng/ml
- Measurement range 0.4 - 40 ng/ml
- Diagnostic specificity 96,7 %
- Diagnostic sensitivity 93,3 %
- Correlation with comparison methods RIA (60 samples; with determination of 7-point calibration curve)
  y = 0.975x-0.731; r^2 = 0.903
- Traceability of ECP calibration system by protein determination of the purified ECP via UV absorption.

*** = of basic ng/ml**

8. Relevant interferences

Icterus	0 – 18.3 mg/dl bilirubin F – no impairment 0 – 19.0 mg/dl bilirubin C – no impairment
Chyle	up to 1390 units (formazine) – no impairment
Rheumatoid factor	0 - 500 IU/ml – no impairment

Also, do not use weakly haemolytic serums!
Do not use highly lipaemic serums!

9. Preparation and storage of specimen

The ECP concentrations can be affected by sampling and sample storage. Under certain circumstances, the release of ECP from eosinophilic granulocytes may also occur on the coagulation of blood. **The following methods for sampling and sample storage must be adhered to.**
Use Vacutainer SST blood sample tubes (with separating gel, e.g. order number 366444) from Becton Dickinson, Heidelberg, or Sarstedt S-Monovette (with serum gel, e.g. order number 02.1388.001) for drawing the blood samples. After placing the blood into the drawing tube, mix the content by stirring it 6 times by 180 °. Any variation of temperature, time or serum vials results in a change to the ECP release and can distort the values. After sampling, allow the blood to stand for **exactly 60 minutes** in a vibration-free location at 20 to 25 °C (RT). Direct exposure to sunlight and the vicinity of heating radiators must be avoided. Immediately afterwards centrifuge the blood for **exactly 10 minutes** at 1200 g (gravitational acceleration g = 9.81 m/s^2).
Immediately after centrifuging, however no more than one hour, transfer the serum to a new vial (glass or polystyrene without separating gel).
For shipping purposes, the serum which is obtained can be stored for 24 hours. If the determination is carried out later, the serum must be frozen at – 20 °C or lower. Avoid repeated freezing and thawing!

10. Test procedure

1. Before starting the test, all components must be brought to room temperature (RT, 20 to 25 °C). This temperature must be maintained during the entire test.
2. All micro-titration strips which are not used immediately must be returned to the original bag, which must be sealed tight in order to prevent absorption of moisture. Used micro-titration strips must be disposed of.
3. Do not expose the test kit to direct sunlight.
4. The micro-titration plate wells should not dry out completely during the test run.
5. There should be no contamination in the wells of the micro-titration plates. Before measurement, it must be ensured that there are no air bubbles in the wells.
6. Preparation of the washing solution: 50 ml of the washing solution concentrate to 1000 ml with distilled water and mix thoroughly.. With a storage temperature of 2 to 8 °C the washing solution concentrate may be slightly cloudy or have a sediment. Take care that the clouding or sediment is completely dissolved when preparing the washing solution. This solution is sufficient for a micro-titration plate with 96 wells. After dilution the washing solution can be stored for 2 weeks at 4 °C if thoroughly cleaned vessels are used.
7. Dilution of the samples: 50 µl serum with 200 µl diluent solution (dilution ratio 1:4). For this, first pipette 50 µl of serum into each well of an **non-coated** micro-titration plate and then add 200 µl of diluent solution. The positions for the blank and the standards must be left out, as subsequently the diluted serums are to be transferred to the same wells of the **coated** micro-titration plate.
   Dilute the control samples using the same procedure as for the patient samples.
8. Then pipette the standards and the **diluted serums** into the **coated** micro-titration strips: Well A1 is used as a blank. This well remains empty. For double determinations, pipette 100 µl of each of the **undiluted standards** 1 - 5 into the corresponding wells (B1 - C2). Then transfer the diluted serums from the non-coated micro-titration plate into the coated plate using an 8-channel pipette. The diluted serums must be transferred to the same positions.

Example for points 7 and 8 (double determination standards - single determination samples):
A non-coated micro-titration plate is used for dilution of the patient serums. The positions A1 for the blank and the positions B1 to C2 for the standards remain free. From positions D2 upwards, pipette 50 µl serum and 200 µl diluent buffer into each well. Then pipetting is carried out in a coated micro-titration plate. Well A1 remains empty (blank). 100 µl of undiluted standard 1 into wells B1 and C1. 100 µl of undiluted standard 2 into wells D1 and E1 etc., up to standard 5, which is pipetted into wells B2 and C2. Then, using an 8-channel pipette 100 µl of diluted serum from the micro-titration plate in which the dilution was performed into the coated micro-titration strip. Here, transfer the diluted serums by pipette from the corresponding micro-titration plate wells, i.e. in the first step, pipette 100 µl of diluted serum out of the wells D2 to H2 into wells D2 to H2 of the coated micro-titration plate (micro-titration strip). In the next step, using the 8-channel pipette, pipette 100 µl of diluted serum out of wells A3 to H3 into wells A3 to H3 of the coated micro-titration plate etc.

9. Cover the micro-titration plate and incubate for 1 hour at room temperature (RT).
10. Wash the wells of the micro-titration plates either with the TECAN Columbus automatic washer (4x) or with the Omega Diagnostics washer (5x). Please observe the operating instructions! Only washing procedures approved by Omega Diagnostics must be used.
11. Pipette 100 µl of the conjugate solution directly into the wells, however not into the blank substrate value. Then cover the micro-titration plate again. Incubate for 60 min at RT.
12. Wash as described under 10.10.
13. Pipette 100 µl substrate solution into all of the wells. Cover the micro-titration plate again and incubate for 10 min without exposure to light.
14. Using the same procedure and sequence as for pipetting the substrate solution, then add 100 µl of stop solution to all of the wells.
15. Measure the micro-titration plate in the photometer at 450 nm. The measures should be obtained within 10 minutes of stopping the reaction.

11. Calculation

1. Calculate the average values of the extinctions of the standard serums.
2. The standard curve can be calculated manually by entering the extinctions determined for the standards against the concentrations of the relevant standard serums (in ng ECP/ml) on semi-logarithmic graph paper and connecting the individual points with a ruler. This standard curve is used to determine the values of the serum samples. The extinctions of the examination samples are compared with those of the standard serums. Note the dilution factor! For dilutions as in Section 10.7, the determined values must be multiplied by 5.
   With Omega Diagnostics devices calculation of the standard curve and the evaluation of the measurement results are carried out automatically. The same evaluation method is used here as for manual methods.

Warning! If significant changes are made to the test procedure (e.g. time, sequence, temperature etc.) or if significant impairment of the analysis performance is seen, even with correct use (e.g. control serum values out of specifications, serious differences in double values etc.) the values which are obtained must not be used. A check of the system or the procedure is essential before continuing work. In case of doubt please contact the specialists at Omega Diagnostics.

12. Normal values for ECP

- Values < 16 ng ECP/ml

(See also Section 6 "Limitations of the procedure" and Reference 1)

13. Warnings and precautions

The following rules must be observed:

1. The relevant safety regulations must be observed when handling the test components.
2. Standards, diluents and examination materials are potentially infectious substances. Suitable agents or methods must be used to disinfect contaminated areas.
3. The stop solution contains sulphuric acid. Wear protective gloves / protective clothing / eye protection / face protection. In case of contact with the skin (or hair): take off all contaminated clothing immediately. Wash or shower the skin with water. In case of contact with the eyes: carefully rinse with water for several minutes. If possible, remove any contact lenses. Continue rinsing. Inform the poison centre or doctor immediately. Wash contaminated clothing before wearing it again.
4. Smoking, eating and drinking are prohibited in the laboratory. Do not ingest!
5. Do not suck the pipette with your mouth!
6. Close all reagents after use. The closures must not be mixed up.
7. Avoid cross-contamination when pipetting!
8. Test components from different batches must not be mixed.
9. Reagents must not be used after the expiry date.
10. Reference samples and kit controls must be included with every assay array performed to ensure correct results.
11. The functionality and accuracy of the equipment used (pipettes, photometer etc.) must be checked at regular intervals. Observe the manufacturer's instructions!
12. Reagents and chemicals must be handled and disposed of according to the applicable regulations.
    List of supplied substances which may require special treatment for disposal:
    - Conjugate (bovine serum albumin CAS 90604-29-8)
    - Substrateon (Tetramethyl benzidine/ H_2O_2; contains organic solvents)
    - Diluent solution (goat serum; sodium azide < 0.1% w/w CAS 26628-22-8)
    - Stop solution (sulphuric acid 0.5 M CAS 7664-93-9)
    - Standard serum (goat serum; sodium azide < 0.1% w/w CAS 26628-22-8)

14. Quality control

- *Internal quality control*
  It is recommended that for each test set at least one positive serum and patient serum are used in the test. If the control is within the normal range, it can be assumed that the test method is functioning adequately.
  The following criteria must be observed:
  Blank value: Extinction <0.10
  Standard 40 ng/ml: Extinction > 1.50
  It is recommended that quality control records are kept.
- *External quality control*
  Participation in external quality controls (ring tests) is recommended. Here, samples with unknown analytical concentrations are not known to the laboratory participating in the external quality control are sent by a ring test provider. After collection of the results, the ring test provider evaluates and assesses the results from all senders. Details must be obtained from the ring test provider. Please contact Omega Diagnostics or your in-vitro sales representative.

15. Storage of the test kit

2 to 8 °C

16. Expiry date

The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Expiry date is the last day of the month on the bottle and the kit label. Do not use reagents after the expiry date.

17. References

1. Gleich, G., et al., Proc. Natl. Acad. Sci. USA, 83, 3146-3150, (1986)
2. Krisyjansson, S., et al., Annals of medicine 28, 395-399, (1996)
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4. Zimmerman, B., et al., Clin. Exp. Allergy, 23, 564-570, (1993)
5. Ren-Bin Tang, et al., Pediatric Pulmology 31, 121-125 (2001)
6. Kirchbach, G. von, et al., Allergologie No. 12, 491-497 (2005)

18. Date of information

These instructions for use are valid from 08.07.2015.

19. Ordering information

	Article number
ECP test kit	REF 36061000
Positive control	REF 36061002

20. Manufacturer

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