Enzyme immunoassay for the quantitative determination of human Eosinophilic Cationic Protein in serum.

3. Substrate
- 1 bottle with 20 ml tetramethylbenzidine/H2O2

4. Stop solution
- 1 bottle with 20 ml 0.5 M sulphuric acid.

5. Diluent solution
- 1 bottle with 50 ml diluent solution; contains 1% goat serum. Preservation of the ECP antibodies.

6. CAL/SE RUM
- Calibration system:
  - 5 bottles, each with 1.3 ml serum with various concentrations of ECP. Contains 1% goat serum.
  - Preservation of the ECP antibodies.

7. MTP
- Micro-titration strips: 12 micro-titration strips (individually removable) each with 8 wells, coated with anti-human ECP antibodies.

5. Additional materials and devices
- Micro-pipette with disposable tips, 50 µl and 100 µl
- Manual hand dispenser e.g. Eppendorf Multipette
- Micro-titration strips
- Adhesive film or micro-titration plate cover
- Micro-titration plates (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 (Becton Dickinson) or 5-Monovette with serum gel (Sarstedt)
- Laboratory centrifuge
- Omega Diagnostics Alpha-/Quattro-System

6. Repeatability
- Intra-assay: < 12 CV %
- Inter-assay: 7% (k=1)

8. Relevant interferences
- Icterus
  - 0 - 18.3 mg/dl bilirubin F
  - No interference
  - 19.0 - 30.0 mg/dl bilirubin C
  - No interference

- Chyle
  - up to 1390 units (formazine)
  - No interference

- Rheumatoid factor
  - 0 - 500 IU/l
  - No interference

- Also, do not use weakly haemolytic sera!
- Do not use highly lipaemic sera!

9. Preparation and storage of specimen
- The ECP concentrations can be affected by sampling and sample storage. In doubtful 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 5-Monovette (with serum gel, e.g. order number 02.1388.001) for drawing the blood samples. After the first bleed 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 measured value. With the values complying, the test should 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 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
- 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 cloudy 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.
- 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 sera 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 sera.
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
conducted 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 solutions
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
Omegas 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
minutes 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 (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 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
Omegas Diagnostics.

12. Normal values for ECP

- Values < 16 ng ECP/ml

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)
- Substrate (Tetramethyl benzidine/ H2O2; 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
3150, (1986)
2. Krisyjansson, S., et al., Annals of medicine 28, 395-399,
(1996)
(1988)
4. Zimmerman, B., et al., Clin. Exp. Allergy, 23, 564-570,
(1993)
5. Ren-Bin Tang, et al., Pediatric Pulmology 31, 121-125
(2001)
(2005)

18. Date of information
These instructions for use are valid from 08.07.2015.

19. Ordering information
ECP test kit Article number AECO-1200 Article number
Positive control AECO-1290 Article number

20. Manufacturer
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