

CE

MESACUP ECP TEST

Cat. No. 7618E: 96 wells

English

1. Intended Use

The MESACUP ECP TEST is quantitative assay kit for human ECP in serum and urine by sandwich ELISA.

2. Summary and Explanation

Eosinophil Cationic protein (ECP), Eosinophil derived Neurotoxin (EDN) and Major Basic Protein (MBP) are known to be major protein-mediators derived from activated eosinophils. ECP and EDN are found in matrix of granules in eosinophils whereas MBP is found in core of granules. ECP and EDN are members of the ribonuclease A superfamily. ECP and MBP have high cytotoxicity. These three proteins are highly cationic proteins with PH 10.8-10.9. Activated eosinophile play an important role in the late asthmatic response and in the asthmatic airway inflammation. As ECP is secreted from activated eosinophils, ECP can be a marker of eosinophile activation and degranulation.

“MESACUP ECP TEST” is for measuring ECP specifically with high sensitivity by ELISA.

3. Principle

MBL MESACUP ECP TEST measures human ECP by sandwich ELISA. This ELISA detects human ECP with a minimum detection limit of 0.125 ng/ml and does not cross-react with EDN.

In the wells coated with anti-human ECP monoclonal antibody, samples to be measured or standards are incubated. After washing, a peroxidase conjugated anti-human ECP polyclonal antibody is added into the microwells and incubated. After another washing, the peroxidase substrate is mixed with the chromogen and allowed to incubate for an additional period of time. An acid solution is then added to each well to terminate the enzyme reaction and to stabilize the developed color. The optical density (O.D.) of each well is then measured at 450 nm using a microplate reader. The concentration of ECP is calibrated from a standard curve based on reference standards.

4. Brief Assay Procedure

Sample Incubation	Add diluted sample 100 μ l Room temperature (20~25°C) 60 min.
	↓
Wash	Wash 4 times
	↓
Conjugate Incubation	Add conjugate solution 100 μ l Room temperature (20~25°C) 60 min.
	↓
Wash	Wash 4 times
	↓
Substrate Incubation	Add substrate reagent 100 μ l Room temperature (20~25°C) 10 min.
	↓
Stop Reaction	Add stop solution 100 μ l

↓
Read Absorbance at 450 nm
↓
Interpretation of Result

5. Materials provided

Materials	Quantity (96 wells)
Microwell strips coated with anti-human ECP antibody	8-well x12 strips
ECP standard (0.09% sodium azide; Preservative, 1% Goat Serum; ready to use)	1.3 ml x 6 vials
Assay diluent (0.09% sodium azide; Preservative, 1% Goat serum)	20 ml x 1 bottle
Conjugate reagent (Horseradish Peroxidase conjugated anti-human ECP polyclonal antibody, 1% BSA; ready to use)	15 ml x 1 bottle
Wash concentrate (10 x concentrated solution)	100 ml x 1 bottle
Substrate reagent (TMB/H ₂ O ₂ ; ready to use)	20 ml x 1 bottle
Stop solution (0.5 mol/L H ₂ SO ₄ ; ready to use)	20 ml x 1 bottle
Instruction manual	1
Positive control (0.09% sodium azide; Preservative, 1% Goat Serum)	1.3 ml x 1 vial
Negative control (0.09% sodium azide; Preservative, 1% Goat Serum)	1.3 ml x 1 vial

6. Materials and equipment required

- Microplate reader
- Plate washer or washing bottle
- Adjustable micropipette
- Multichannel micropipette
- 96-well polyvinyl plate (for preparation of first reaction)
- Reagent reservoir
- Distilled water
- One liter graduated cylinder for preparation of wash solution

7. Analytical Precautions

1. All breakaway microwell strips which are not immediately required should be returned to the ziplock pouch which must be carefully resealed to avoid moisture absorption. Wells which have been processed must be discarded.
2. Do not use kit components beyond the stated expiration dates.
3. Do not expose the kit to direct sun during assay and storage.
4. Avoid microbial and cross contamination of reagents or samples. Cross contamination of reagents and /or sample could cause false results.

5. Incubation temperatures above or below normal room temperature (20~25°C), shorter or longer time periods of incubation and inaccurate dilution may give erroneous results. All reagents must be brought to 20~25°C before starting the assay.
6. In washing steps, the wells must be rinsed with wash solution properly enough to avoid false positive.
7. Carefully pipette not to foam each sample and reagent to avoid contamination of each microwell.
8. Assay diluent may form precipitate, which does not cause inconsistent results.
9. When wash concentrate is stored at 2~8°C, some precipitation or turbidity may appear. However it does not affect the reagent efficiency. Dissolve the wash concentrate completely when preparing the wash solution. Prior to preparation of wash solution, dissolve thoroughly.
10. Contamination of the TMB substrate solution with conjugate or other oxidants will cause the solution to change color prematurely.
11. Do not allow wells to become dry during an assay procedure.
12. Ensure that the bottom of the plate is clean and dry, and no air bubbles are present on the surface of the liquid in the wells before reading the plate.
13. This product is for in vitro diagnostic use only.

8. Health and Safety Information

1. Positive control and Negative control are derived from human serum, in which HBs antigen, HCV antibody, HIV-1 and HIV-2 antibodies has not been detected. No test method, however, can guarantee the absence of these or any other infectious agents. These reagents and all patient samples should be handled as if they are capable of transmitting AIDS, hepatitis or any other infectious diseases.
2. Some kit components contain animal origin materials, which are from non-infectious animals. These components, however, should be treated as potential biohazards in use and for disposal.
3. Avoid contact of reagents with eyes, skin and clothing. Reagents on skin must be washed away with plenty of water. TMB contains irritant and Stop Solution consists of 1N sulfuric acid, which is a poison and corrosive.
4. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
5. Liquid waste containing acid must be neutralized with base, e.g. sodium bicarbonate, before decontamination with sodium hypochlorite. Acid-containing liquid waste that has been neutralized and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0 %. A 30 minute exposure to 1.0 % sodium hypochlorite is necessary to ensure effective decontamination.
6. ECP standard, Positive control, Negative control, and Assay diluent contain sodium azide (0.09%) as a preservative. Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush with plenty of water when disposing materials containing azide into a drain.

9. Procedure

■ Preparation of Reagents

Wash solution: Dilute 100 ml of Wash concentrate with 900 ml of distilled water. The diluted wash solution is stable for 2 weeks at 4 °C

■ Preparation of samples

1) Sampling

Serum levels of ECP can be varied by *in vitro* effect after sampling. There is a possibility that *in vitro* release of ECP from eosinophils can occur during coagulation. Therefore, each laboratory is recommended to establish its own sampling procedure. The following sampling procedure has been reported.

<Serum>

Blood for measurement of ECP was collected in Vacutainer SST tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, N.J., USA). The blood allowed to clot at room temperature for exactly 60 minutes. Thereafter, the serum was separated by centrifugation for 10 minutes at 1200 x g, and then transferred to a new test tube and stored at -20 °C until the analyses were performed.

<Urine>

Urine was collected from each individuals as a spot sample and immediately frozen at -20 °C. Urinary creatinine also quantitated and urinary ECP results were presented as µg ECP/m mol creatinine.

2) Dilution of sample

Dilute each sample with Assay diluent.

Human serum: 1:5 with Assay diluent. Ex. by adding 50 µl of sample to 200 µl of Assay diluent.

Human urine: 1:2 with Assay diluent. Ex. by adding 150 µl of sample to 150 µl of Assay diluent.

Positive control: 1:5 with Assay diluent. Ex. by adding 50 µl of sample to 200 µl of Assay diluent.

Negative control: 1:5 with Assay diluent. Ex. by adding 50 µl of sample to 200 µl of Assay diluent.

3) Storage

Fresh samples should be used. Aliquote each sample into new plastic tube, and store below -20 °C if necessary. Do not repeat freezing and thawing.

■ Assay procedure

STEP 1. (Sample incubation)

- (1) Add 150 µl of prepared samples and standards (do not dilute standard) to 96-well polyvinyl preparation plate as the same order of assay run. Then transfer 100 µl of each sample to the antibody coated microwell using multichannel pipet and mix well.
- (2) Incubate for 60 minutes at room temperature (20~25 °C).

STEP 2. (Washing)

Aspirate or discard the well contents. Fill the well with Wash solution and then completely aspirate or discard the contents. Wash the well 4 times with wash solution using washing bottle. When autowasher is used, wash 4 times.

* Each laboratory is recommended to confirm its own appropriate washing times and set-up.

* Washing buffer should be used at room temperature (20~25°C).

*Remove excess Wash solution by gentle tapping or aspiration.

STEP 3. (Conjugate incubation)

- (1) Pour conjugate reagent (ready to use) into a reservoir. After removing wash solution completely, pipette 100 µl of Conjugate reagent to each well with multichannel pipette.
- (2) Incubate for 60 minutes at room temperature (20~25°C).

STEP 4. (Washing)

Wash the microplate following the STEP2.

STEP 5. (Substrate incubation)

- (1) Pour Substrate reagent into the reagent reservoir. After removing wash solution completely, pipette 100 μ l of Substrate reagent to each well with multichannel pipette.
- (2) Incubate for 10 minutes at room temperature (20~25°C).

STEP 6. (Stopping reaction)

Pour Stop solution into a reservoir. Pipette 100 μ l of stop solution to each well with multichannel pipette.

STEP 7. (Reading)

Read the absorbance of each well at 450 nm. If a dual wavelength plate reader is available, set the test wave length at 450 nm and the reference at 620 nm.

*Reading should be done within 30 minutes after stopping the reaction.

STEP 8. (Calculation of results)

Calculate the mean absorbance value of each standard. Plot on semi-log graph paper and construct a standard curve [Absorbance on the vertical axis, concentration (in ng/ml) on the horizontal axis].

If using a program to automatically calculate the concentration, it is recommended that the best fitting curve is used.

Report the ECP concentration of samples by multiplying the value read from the standard curve by dilution factor (e.g. x 5).

*An international reference material for ECP is not available. ECP was purified and measured its protein concentration by Ultraviolet absorbance method and protein measurement method.

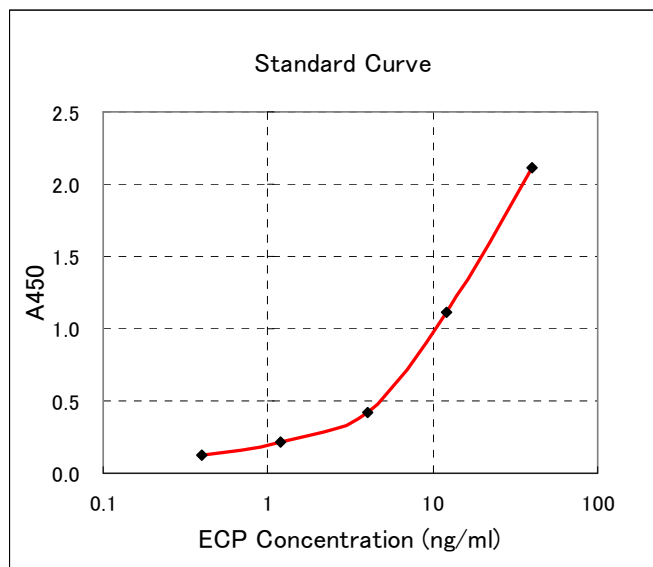
■ Concentration of Human ECP in normal human serum.

Serum sample from healthy blood donors were assayed by the MESACUP ECP TEST.

148 healthy donor sera were measured. After removing those samples measuring over the mean + 3SD, the new mean + 3SD was determined to be: mean + 3SD = 15.6 ng/ml.

Sample n =148 mean= 4.06 ng/ml SD=3.86 mean+3SD=15.6 ng/ml

■ Example of standard curve



■ Quality Control

Each assay result should meet the following criteria.

A450 of reagent blank : < 0.10

A450 of ECP Standard 50ng/ml : > 1.5

The Positive and Negative Controls must give the following results:

	Positive Control	Negative Control
ECP value (ng/ml)	20<, <40	<2

If any of these are not met, the results are invalid and the test should be repeated.

Before repeating assay, check the following procedure.

- Incubation Temperature
- Incubation Period of Time
- Washing

10. Performance Characteristics

■ Sensitivity

The sensitivity of the assay is 0.125 ng/ml.

■ Reproducibility

1. Intra-assay

Intra-assay reproducibility was determined by assaying the sample 8 times.

Sample	Sample 1	Sample 2	Sample 3	Sample 4
Number of determinations	8	8	8	8
Mean (ng/ml)	1.35	1.51	4.96	6.10
C.V.(%)	6	5	6	5

2. Inter-assay

Inter-assay reproducibility was determined by 6 independent assays of the sample*.

Sample	Sample 1	Sample 2	Sample 3
Number of determinations	4	4	4
Mean (ng/ml)	1.66	4.73	5.82
C.V.(%)	10	6	5

*From eight (8) replicates of each serum sample in six (6) separate assays.

3. Lot-to-lot

Lot-to-lot assay reproducibility between three (3) different lot.

Sample	Sample 1	Sample 2	Sample 3	Sample 4
Number of lots	3	3	3	3
Mean (ng/ml)	24	1.50	4.62	6.07
C.V.(%)	4.5	4.9	3.8	3.31

■ Recovery test

Purified ECP was added to three different samples at different concentrations.

Sample 1

(A) Additional ECP (ng/ml)	ECP concentration observed (ng/ml)	(B) Recovery (ng/ml)	(B/A) Recovery (%)
0	10.57	-	-
2.5	13.39	2.82	112.8
5	14.69	4.12	82.4
10	19.72	9.15	91.5

Sample 2

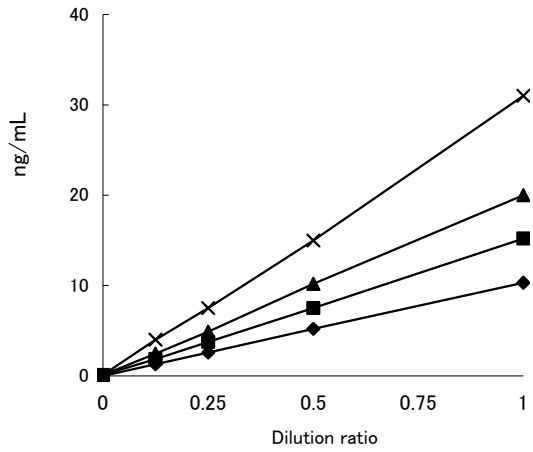
(A) Additional ECP (ng/ml)	ECP concentration observed (ng/ml)	(B) Recovery (ng/ml)	(B/A) Recovery (%)
0	5.05	-	-
2.5	7.66	2.61	104.4
5	10.59	5.54	110.8
10	15.88	10.83	108.3

Sample 3

(A) Additional ECP (ng/ml)	ECP concentration observed (ng/ml)	(B) Recovery (ng/ml)	(B/A) Recovery (%)
0	1.78	-	-
2.5	4.34	2.56	102.4
5	6.99	5.21	104.2
10	13.04	11.26	112.6

■ Dilution test

Sample was diluted with Assay diluent.

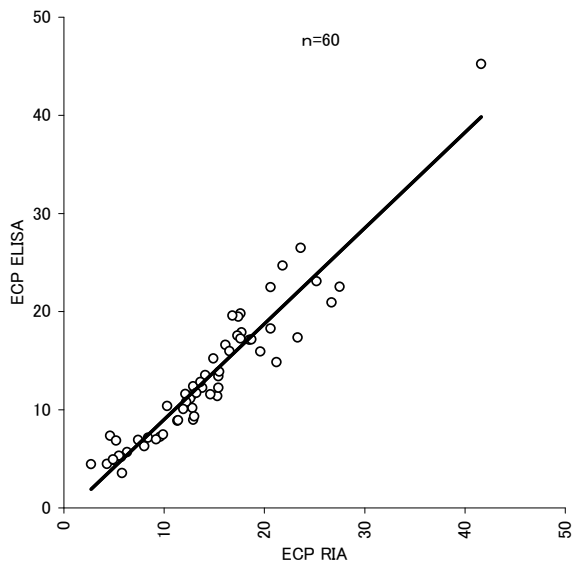


■ **Correlation between RIA test and MBL MESACUP ECP TEST**

60 samples were measured by RIA test and MBL MESACUP ECP TEST.

$$Y=0.975x-0.731$$

$$r^2=0.903$$



■ **Interfering substances**

Bilirubin F (up to 18.3 mg/dl), Bilirubin C (up to 19.0 mg/dl), Chyle (up to 1390 unit as Formazine) and /or Rheumatoid factor (up to 500 IU/ml) are not affective on the assay result, but avoid using highly lipemic samples.

Hemoglobin does affect the assay results.

No effect was found to assay values in adding up to 500ng/mL of EDN.

■ **Assay Range**

The assay range of this kit is from 0.125ng/nl to 40 ng/ml.

■ **Package size**

96 wells

■ **Storage and Stability**

All kit components must be stored at 2-8°C. All reagents are stable for 12 months after manufacturing when stored at the conditions indicated.

11. References:

1. Gleich, G., et al., Proc. Natl. Acad. Sci. USA, 83, 3146-3150 (1986)
2. Krisyjanon, S., et al., Annals of medicine 28, 395-399, (1996)
3. Peterson, C., et al., Eur. J Haematol., 40, 415-423 (1988)
4. Zimmerman, B., et al., Clin Exp Allergy, 23, 564-570 (1993)

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