HUMAN EOSINOPHIL CATIONIC PROTEIN (ECP) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN EOSINOPHIL CATIONIC PROTEIN (ECP) CONCENTRATIONS IN SERUM AND EDTA PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN EOSINOPHIL CATIONIC PROTEIN (ECP) ELISA
Catalog No.	SK00128-01
Lot No.	
Formulation	96 T
Standard range	0.3125-20 ng/ml
Sensitivity	0.05 ng/ml
Sample require	100 µl
Dilution Factor	*Optimal dilutions should be determined by each laboratory for each application*
Sample Type	Serum, EDTA Plasma
Specificity	Human Eosinophil Cationic Protein (ECP)
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2-8°C

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Human Eosinophil Cationic Protein (ECP) immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Human Eosinophil Cationic Protein (ECP) in serum and plasma. It contains Human Eosinophil Cationic Protein (ECP) from Human Eosinophils and antibodies raised against recombinant Human ECP protein. Results obtained with naturally occurring Eosinophil Cationic Protein (ECP) samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural Human Eosinophil Cationic Protein (ECP).

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Eosinophil Cationic Protein (ECP) has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Eosinophil Cationic Protein (ECP) present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for Eosinophil Cationic Protein (ECP) is added to the wells. Following a wash to remove any unbound antibody reagent, a Streptavidin-HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of Eosinophil Cationic Protein (ECP) bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Eosinophil Cationic Protein (ECP) Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against Eosinophil Cationic Protein (ECP).	**128-01-01**	**1 plate**
Eosinophil Cationic Protein (ECP) Standard – 40 ng/vial of Human Eosinophil Cationic Protein (ECP) in a buffered protein base with preservatives; lyophilized.	**128-01-02**	**1 vial**
Detection Antibody– 105uL/vial, 100-fold concentrated of a purified antibody against Eosinophil Cationic Protein (ECP) with preservatives; lyophilized.	**128-01-03**	**1 vial**
Positive Control – one vial of Human Eosinophil Cationic Protein (ECP), lyophilized	**128-01-04**	**1 vial**
Streptavidin HRP Conjugate - 160 µl/vial, 75-fold concentrated solution of Streptavidin-HRP conjugate	**SAHRP**	**1 vial**
Standard Reconstitute Solution – 1.5mL of solution	**DB02A**	**1 vial**
Dilution Buffer – 25 mL of buffered protein based solution with preservatives	**DB300**	**1 bottle**
Antibody Diluent Solution - 12 mL of buffered protein based solution with preservatives	**DB08**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservatives	**DB01**	**1 bottle**
Wash Buffer - 50 ml of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 ml of TMB substrate solution	**TMB01**	**1 bottle**

Stop Solution - 11 ml of 0.5M HCL	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20 °C or -70°C for up to one month. Streptavidin-HRP Conjugate 75-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Plasma samples may require 4-8 fold dilution. A suggested 4-fold dilution is 80 µL sample + 240 µL Dilution Buffer (DB300). A suggested 8-fold dilution is 40 µL sample + 280 µL Dilution Buffer (DB300). Serum samples may require 10-20 fold dilution. A suggested 10-fold dilution is 30 µL sample + 270 µL Dilution Buffer (DB300). A suggested 20-fold dilution is 15 µL sample + 285 µL Dilution Buffer (DB300).

Optimal dilutions should be determined by each laboratory for each application. It is very important to pretest the sample dilution before performing the final assay.

Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

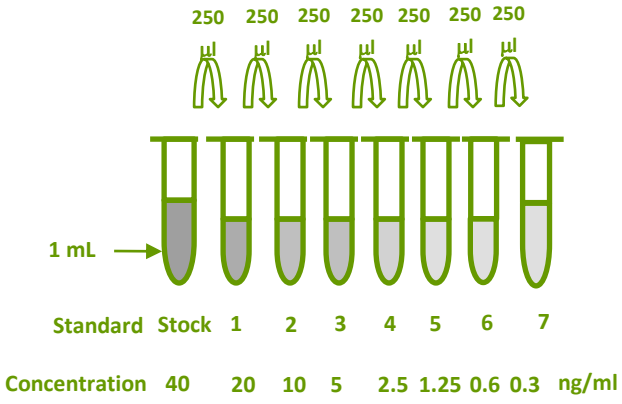
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

Eosinophil Cationic Protein (ECP) Standard - Refer to vial label for reconstitution volume. Reconstitute the **Eosinophil Cationic Protein (ECP)** Standard with 1.0 mL of **Standard Reconstitute Solution** (DB02A). This reconstitution produces a stock solution of 40 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of **Dilution Buffer** (DB300) into tubes #1 to #7. Use the stock solution (40

ng/mL) to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20 ng/mL standard serves as the high standard. The Dilution Buffer (DB300) serves as the zero standard (0 ng/mL). **Cannot use Antibody Diluent Solution or HRP Diluent Solution to reconstitute Standard or for its dilution.**

TUBE	STANDARD	STANDARD RECONSTITUTE SOLUTION	CONCENTRATION
stock	powder	1 ml	40 ng/ml
TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
# 1	250µl of stock	250µl	20 ng/ml
# 2	250µl of 1	250µl	10 ng/ml
# 3	250µl of 2	250µl	5 ng/ml
# 4	250µl of 3	250µl	2.5 ng/ml
# 5	250µl of 4	250µl	1.25 ng/ml
# 6	250µl of 5	250µl	0.625 ng/ml
# 7	250µl of 6	250µl	0.3125 ng/ml

***Human Eosinophil Cationic Protein (ECP) is highly purified from Human Eosinophils which have been tested for infectious diseases. It has been verified non-infectious, but for complete assuarance that infectious agents are absent, this material should be handled at bio-safety level 2 (BSL-2).**



Detection Antibody - Reconstitute the Detection Antibody concentrated with 105 µL of **Antibody Diluent Solution** (DB08) to produce a 100-fold concentrated stock solution. Transfer 105 µL of 100-fold concentrated stock solution to 10.395 mL of **Antibody Diluent Solution** (DB08) to prepare working solution.

Streptavidin-HRP Conjugate - Transfer 160 µl of 75-fold concentrated **Streptavidin-HRP conjugate** stock solution to 11.84 mL of **HRP Diluent Solution** (DB01) to prepare working solution. Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

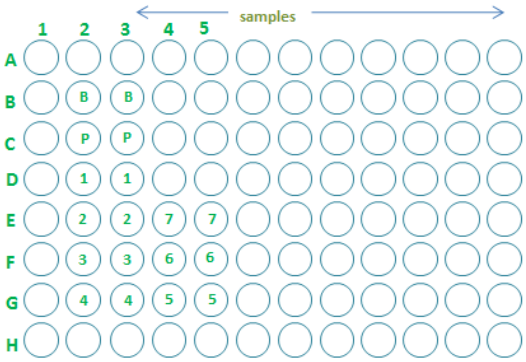
Positive Control- Reconstitute the **Positive Control** with 400 µL of **Standard Reconstitute Solution** (DB02A) for 5-fold concentrated solution. Pipet 60 µL of 5-fold concentrated solution into 240 µL of **Dilution Buffer** (DB300). Note: Positive Control should be prepared and used immediately.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (B2, B3).
4. Add 100 µL of Standard solution from #7 to #1 (reverse order of serial dilution) (E4, E5 to G4, G5 and G2, G3 to D2, D3), sample, or positive control per well (C2, C3). Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**

9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 6-9 minutes at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Eosinophil Cationic Protein (ECP) concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding that of standard 20ng/ml may result in inaccurate, low human Eosinophil Cationic Protein (ECP) levels. Such samples require further external predilution according to expected human Eosinophil Cationic Protein (ECP) values with Dilution Buffer in order to precisely quantitate the actual human Eosinophil Cationic Protein (ECP) level.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED*)
Blank	0 (0.107)
0.156	0.032
0.313	0.069
0.625	0.130
1.25	0.219
2.5	0.338
5	0.540
10	0.808
20	1.190

- Positive Control: 2 – 5.5 ng/mL

CALIBRATION

This immunoassay is calibrated against a highly purified Human Eosinophil Cationic Protein (ECP) from Human Eosinophils.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of Eosinophil Cationic Protein (ECP) was 0.05 ng/mL.

SPECIFICITY

This assay recognizes natural human Eosinophil Cationic Protein (ECP). The factors listed below were prepared at 2000 ng/mL in Dilution Buffer, and assayed for cross reactivity.

PROTEIN NAME	CROSS-REACTIVITY (%)
Human Eosinophil Cationic Protein (ECP), from Human Eosinophils	**100**
Human Eosinophil Cationic Protein (ECP), *E. coli* derived recombinant	**1-2**
Human SPARC	**0**
Human Fetuin A	**0**
Human CRP	**0**
Human NGAL	**0**

LINEARITY

To assess the linearity of the assay, pooled research human plasma samples were diluted with Dilution Buffer (DB300) and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
4x	2.002	8.008	100
8x	0.911	7.288	91

To assess the linearity of the assay, pooled research human serum samples were diluted with Dilution Buffer (DB300) and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
10x	3.138	31.38	100
20x	1.697	33.94	108

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

↓

Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on plate shaker at RT.

↓

Aspirate and wash 4 times.

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Add 100 µl Detection Antibody Working Solution to each well. Incubate 2 hours on the plate shaker at RT.

↓

Aspirate and wash 4 times.

↓

Add 100 µl Streptavidin-HRP conjugate Working Solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**

↓

Aspirate and wash 4 times.

↓

Add 100 µl Substrate Solution to each well. Incubate 6-9 min on the plate shaker. **Protect from light.**

↓

Add 100 µl Stop Solution to each well. Read 450nm within 15 min