HUMAN EOSINOPHIL CATIONIC PROTEIN (ECP) ELISA KIT

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

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

ELISA NAME	**HUMAN EOSINOPHIL CATIONIC PROTEIN (ECP) ELISA**
Catalog No.	SK00128-01
Lot No.	
Formulation	96 T
Standard range	0.156-10 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)
Calibration	Human Eosinophil Cationic Protein (ECP) from human Eosinophils
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Eosinophil Cationic Protein (ECP) ELISA Kit contains the necessary components required for the quantitative measurement of human ECP from serum and EDTA plasma in a sandwich ELISA format.

This immunoassay contains human Eosinophil Cationic Protein (ECP) from human Eosinophils and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural ECP samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human ECP. The capture antibody can bind to the human ECP in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against ECP is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human ECP bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS 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 – 20 ng/vial of human Eosinophil Cationic Protein (ECP) in a buffered protein base with preservative; lyophilized.	**128-01-02**	**1 vial**
Detection Antibody– 1.05 mL/vial, 10-fold concentrate of a biotinylated antibody against Eosinophil Cationic Protein (ECP) with preservative; 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 - 120 µl/vial, 100-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Standard Reconstitute Solution – 1.5 mL of solution	**DB02A**	**1 vial**
Dilution Buffer – 25 mL of buffered protein based solution with preservative.	**DB300**	**1 bottle**
Antibody Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB18**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB06**	**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 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution and Detection Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrated solution and substrate solution can be stored at 2 – 8° C for up to 6 months. (**Protect from light and do not freeze**).
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

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.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and 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).
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

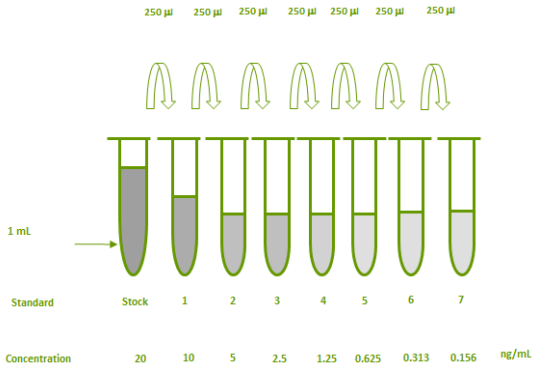
Bring all reagents to room temperature before use.

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 1x Wash Buffer.

Eosinophil Cationic Protein (ECP) Standard - Reconstitute the Eosinophil Cationic Protein (ECP) standard with 1.0 mL of **Standard Reconstitute Solution (DB02A)**. This reconstitution produces a stock solution of 20 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 (20 ng/mL) to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **10 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 and HRP Diluent Solution to reconstitute Standard or for its dilution.**

TUBE	STANDARD	STANDARD RECONSTITUTE SOLUTION	CONCENTRATION
stock	powder	1 ml	20 ng/ml
TUBE	**STANDARD**	**DILUTION BUFFER**	**CONCENTRATION**
# 1	250µl of stock	250µl	10 ng/ml
# 2	250µl of 1	250µl	5 ng/ml
# 3	250µl of 2	250µl	2.5 ng/ml
# 4	250µl of 3	250µl	1.25 ng/ml
# 5	250µl of 4	250µl	0.625 ng/ml
# 6	250µl of 5	250µl	0.3125 ng/ml
# 7	250µl of 6	250µl	0.156 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).**



Positive Control - Reconstitute the Positive Control with 1 mL of **Standard Reconstitute Solution (DB02A)** for 2-fold concentrated solution. Pipet 120 µL of 2-fold concentrated solution into 120 µL of **Dilution Buffer (DB300)**. **Note:** Positive Control concentrated solution could be reused within a few days if stored at -20° C or -70° C.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB18)** to produce a 10-fold concentrated stock solution. Transfer 1.05 mL of 10-fold concentrated stock solution to 9.45 mL of Antibody Diluent Solution (DB18) to prepare working solution.

Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated Streptavidin-HRP conjugate stock solution to 11.88 mL of **HRP Diluent Solution (DB06)** to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days **(protect from light)**.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of Dilution Buffer to Blank well.
4. Add 100 µL of Standard dilutions from #7 to #1 (reverse order of serial dilution), sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x 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 plate sealer. Incubate for 2 hours on microplate 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 microplate 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 3-5 minutes on microplate shaker 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 microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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

TYPICAL STANDARD CURVE

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 (0.143)
0.156	0.107
0.313	0.262
0.625	0.445
1.25	0.578
2.5	0.920
5	1.270
10	1.567

- Lot No.:
- Positive Control:

SPECIFICITY

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 dilutions, samples, or positive control to the well. Incubate 2 hours on plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
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 3-5 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.