HUMAN SOLUBLE E-CADHERIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE E-CADHERIN CONCENTRATIONS IN SERUM AND HEPARIN PLASMA

PRODUCT INFORMATION:

THIS IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN SOLUBLE E-CADHERIN ELISA**
Catalog No.	SK00094-01
Lot No.	
Formulation	96 T
Standard Range	187.5 – 12,000 pg/mL
Sensitivity	40 pg/mL
Sample Volume	100
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum and heparin Plasma
Specificity	Human E-Cadherin only
Calibration	Human E-Cadherin recombinant
Intra-assay Precision	6 - 8%
Inter-assay Precision	10 - 12%
Storage	2 – 8° C for 1 month. More information check page 2-3
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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.

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DESCRIPTION

This Human Soluble E-Cadherin ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human E-Cadherin from serum and heparin plasma in a sandwich ELISA format.

This immunoassay contains recombinant human E-Cadherin and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural E-Cadherin samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human E-Cadherin. The capture antibody can bind to the human E-Cadherin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human E-Cadherin 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 E-Cadherin 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
E-Cadherin Microplate - 96 well polystyrene microplate coated with an antibody against E-Cadherin.	**094-01-01**	**1 plate**
E-Cadherin Standard – refer to lot of recombinant human E-Cadherin in a buffered protein base with preservative; lyophilized.	**094-01-02**	**1 vial**
Detection Antibody Concentrate – refer to lot of biotinylated antibody against E-Cadherin with preservative; lyophilized.	**094-01-03**	**1 vial**
Positive Control - one vial of recombinant human E-Cadherin; lyophilized.	**094-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer - 40 mL of buffered protein based solution with preservative.	**DB300**	**1 bottle**
Antibody & HRP Diluent Solution – 25 mL of buffered protein based solution with preservative; lyophilized.	**DB01**	**1 tube**
Antibody Diluent Solution Concentrate – 11 mL of buffered protein based solution with preservative; lyophilized.	**DB20**	**1 tube**
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**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. For longer storage for up to 8 months, unopened Standard, Positive Control, Antibody Concentrate,

Dilution Buffer, Antibody & HRP Diluent Solution and Antibody Diluent Solution Concentrate should be stored at -20° C. Do not use kit past expiration date. Streptavidin-HRP Conjugate 100-fold concentrated solution **(protect from light)** and **TMB Substrate Solution** should be stored at 2 – 8° C only for up to 8 months. Do not use kit past expiration date.

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.

SAMPLE COLLECTION AND STORAGE

Plasma - Collect plasma using heparin 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. *** *EDTA plasma is not suitable for use in human E-cadherin assay.*

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.

SAMPLE PREPARATION

Serum and Heparin Plasma samples may require 100 dilutions by **Dilution Buffer DB300**. A suggested 100-fold dilution is 5 µL sample + 495 µL Dilution Buffer.

Note: Aviscera Bioscience's Dilution Buffer DB300 is necessary to stabilize the structure of soluble E-Cadherin immunoreactive with capture antibody pre-coated on microplates.

Optimal dilutions should be determined by each laboratory for each application with a pretest.

Use polypropylene test tubes.

REAGENT PREPARATION

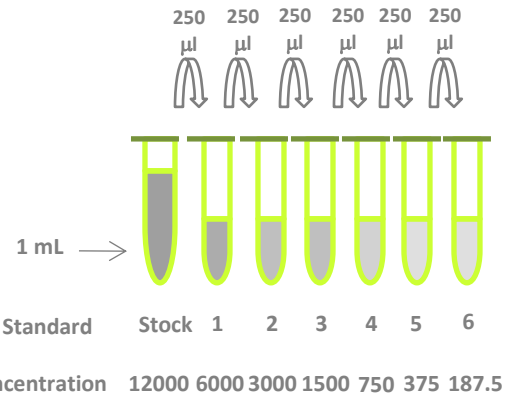
Bring all reagents to room temperature before use.

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.

Antibody Diluent Solution Concentrate – Reconstitute the Antibody Diluent Solution Concentrate with 11.0 mL of **Antibody & HRP Diluent Solution (DB01)** in provided 15 mL centrifuge tube to prepare **Antibody Diluent Solution (DB20)**.

E-Cadherin Standard - Reconstitute the E-Cadherin standard with refer to lot of Dilution Buffer (DB300). This reconstitution produces a stock solution of 12,000 pg/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 into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **12,000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	Refer to lot	12000 pg/mL
# 1	250 µL of stock	250 µL	6000 pg/mL
# 2	250 µL of 1	250 µL	3000 pg/mL
# 3	250 µL of 2	250 µL	1500 pg/mL
# 4	250 µL of 3	250 µL	750 pg/mL
# 5	250 µL of 4	250 µL	375 pg/mL
# 6	250 µL of 5	250 µL	187.5 pg/mL



Positive Control - Reconstitute the Positive Control with refer to lot of Dilution Buffer (DB300).

Detection Antibody - Reconstitute the Detection Antibody with refer to lot of **Antibody Diluent Solution (DB20)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody Diluent Solution (DB20)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. **Note:** ***Prepare and incubation one hour at room temperature prior to use.***

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **Antibody & HRP Diluent Solution (DB01)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of streptavidin-HRP should be used same day **(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 of Dilution Buffer to Blank wells.
4. Add 100 µL of **Standard dilutions** in reverse order of serial dilution, **samples,** or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. **Prepare Detection Antibody working solution for one hour prior to use.**
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 refer to lot 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

Average the duplicate readings for each standard dilution, 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 E-Cadherin 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.

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (refer to lot)
187.5	0.053
375	0.090
750	0.164
1500	0.299
3000	0.559
6000	0.982
12000	1.882

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Soluble E-Cadherin	100
Mouse Soluble E-Cadherin	1.2
Human N-Cadherin	0
Human P-Cadherin	0
Human VE-Cadherin	0
Mouse P-Cadherin	0

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 the plate shaker at RT. **Prepare Detection Antibody working solution one hour prior to use.**
↓
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 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate refer to lot on the plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read 450nm within 15 minutes.