HUMAN FOLLICULAR DENDRITIC CELL SECRETED PROTEIN (FDC-SP) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN FDC-SP RECOMBINANT PROTEIN DERIVED FROM E. COLI

THIS PROTOCOL IS PROVIDED FOR DEMONSTRATION ONLY. MORE INFORMATION IN THE LOT SPECIFIC.

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN FDC-SP ELISA
Catalog No.	SK00319-01
Lot No.	
Formulation	5 X 96 T
Standard range	50-800 ng/ml
Sensitivity	Lot specific
Sample require	100 µl
Dilution Factor	*Optimal dilutions should be determined by each laboratory for each application*
Sample Type	Recombinant FDC-SP Recombinant derived from E. Coli
Specificity	Human FDC-SP derived from E. Coli
Intra-assay Precision	8-12%
Inter-assay Precision	8-14%
Storage	4 °C

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INTRODUCTION

Human FDC-SP Immunoassay is a solid phase ELISA designed to measure Human FDC-SP recombinant derived from E. Coli the samples. It contains recombinant Human FDC-SP and antibodies raised against this protein. It has been shown to accurately quantitie recombinant Human FDC-SP. Results obtained with naturally occurring FDC-SP 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 FDC-SP.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for FDC-SP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any FDC-SP present is bound by the immobilized antibody. After washing away any unbound substances, a polyclonal antibody specific for FDC-SP is added to the wells. Following a wash to remove any unbound antibody reagent, a secondary antibody HRP conjugate is add 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 FDC-SP 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
FDC-SP Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified monoclonal antibody against FDC-SP.	**319-01-01**	**1 plate**
FDC-SP Standard – lot specific of recombinant Human FDC-SP in a buffered protein base with preservatives; lyophilized.	**319-01-02**	**1 vial**
Detection Antibody– lot specific of 10-fold concentrated of a purified polyclonal IgG against FDC-SP with preservatives; lyophilized.	**319-01-03**	**1 vial**
Positive Control – one vial of recombinant FDC-SP , lyophilized	**319-01-04**	**1 vial**
Anti Rabbit IgG-HRP Conjugate -120 µl/vial, 100-fold concentrated solution of Goat anti Rabbit IgG conjugate to HRP	**ARIGHRP**	**1 vial**
Dilution Buffer- 50 mL/vial of buffered protein based solution with preservatives	**DB08A**	**1 vial**
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 vial**
TMB Substrate Solution-11 ml / vial of TMB substrate solution	**TMB01**	**1 vial**
Stop Solution（0.5M HCL）, 11 ml /vial of 0.5M HCL	**S-STOP**	**1 vial**
Plate Sealer.	**EAPS**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to lot specific months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrated as well as Dilution Buffer BD08A

should be stored at -20 or -70 °C. Do not use past kit expiration date.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. 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.

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.
FDC-SP Standard - Refer to vial label for reconstitution volume. Reconstitute the **FDC-SP** Standard with lot specific of Dilution Buffer. Pipette 250 µL of the appropriate Dilution Buffer into the tube #2 to #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 800 ng/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

STANDARD	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	Lot specific	
# 1	Lot specific	Lot specific	800 ng/ml
# 2	250µl of 1	250µl	400 ng/ml
# 3	250µl of 2	250µl	200 ng/ml
# 4	250µl of 3	250µl	100 ng/ml
# 5	250µl of 4	250µl	50 ng/ml

Detection Antibody- Reconstitute the **Detection Antibody concentrated** with lot specific of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette lot specific mL of the appropriate Dilution Buffer into the 15 mL centrifuge tube and transfer lot specific mL of 10-fold concentrated stock solution to prepare working solution.

Anti -Rabbit IgG-HRP Conjugate - Transfer 120 µl of 100-fold concentrated **Anti-Rabbit IgG-HRP conjugate** stock solution to 12 mL of **Dilution Buffer** to prepare working solution. *Note: 1 x working solution of Anti-Rabbit IgG HRP Conjugate should be used within a few days.*

Positive Control- Reconstitute the **Positive Control** with lot specific of Dilution Buffer. *Positive Control should be prepared and used immediately*. Reconstituted Positive Control CAN NOT BE REUSED.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

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 foil pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (A2, A3).
4. Add 100 µL of Standard solution from #5 to 1 (reverse order of serial dilution) (from B2 to F3), sample, or positive control per well (G2, G3). Cover with the 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 **Anti Rabbit IgG-HRP Conjugate** working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for lot specific 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, 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 FDC-SP 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 800 ng/ml may result in inaccurate, low human FDC-SP levels. Such samples require further external predilution according to expected human FDC-SP values with Dilution Buffer in order to precisely quantitate the actual human FDC-SP level.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant Human FDC-SP derived from E. Coli.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of FDC-SP Was lot specific..

SPECIFICITY

PROTEIN NAME	CROSS-REACTIVITY
Human FDC-SP	100%
Human sRAGE	0

The recombinant Human FDC-SP derived from HEk293 or CHO or sf21 cells may not be detected by this elisa kit.

TYPICAL DATA

These standard curves* are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)
50	**0.051**
100	**0.103**
200	**0.178**
400	**0.349**
800	**0.787**

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 100µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Detection Antibody to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Anti Rabbit IgG HRP conjugate to each well. Incubate 60 min on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate to each well. Incubate lot specific min on the bench top. Protect from light.
⬇
Add 100 µl Stop Solution to each well. Read 450nm within 15 min