HUMAN WIF-1 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN WIF-1 CONCENTRATIONS IN CELL CULTURE SUPERNATES, PLASMA AND SERUM

PURCHASE INFORMATION:

ELISA NAME	HUMAN WIF-1 ELISA
Catalog No.	SK00508-01
Lot No.	
Formulation	96 T
Standard Range	31.25 - 2000 pg/ml
Sensitivity	10 pg/ml
Sample Volume	100 µl
Sample Type	Cell Culture Supernates, Plasma and Serum
Specificity	Human WIF-1
Sample Dilution	Optimal dilutions should be determined by each laboratory for each application
Intra-assay Precision	4 - 8%
Inter-assay Precision	8 - 12%
Storage	2°C - 8°C

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

Order Contact:
AVISCERA BIOSCIENCE INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

INTRODUCTION

Human WIF-1 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Human WIF-1 in cell culture supernates, plasma and serum. It contains recombinant Human WIF-1 and antibodies raised against this protein. It has been shown to accurately quantify recombinant Human WIF-1. Results obtained with naturally occurring WIF-1 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 WIF-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for WIF-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any WIF-1 present is bound by the immobilized antibody. After washing away any unbound substances, a monoclonal biotinylated antibody specific for WIF-1 is added to the wells. Following a wash to remove any unbound Antibody, 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 WIF-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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

_ 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 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
Human WIF-1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against WIF-1.	**508-01-01**	**1 plate**
WIF-1 Standard – 1000 pg/vial of recombinant Human WIF-1 in a buffered protein base with preservatives; lyophilized.	**508-01-02**	**2 vials**
Detection Antibody Conjugate – 105 µL / vial, 100-fold concentrated of monoclonal antibody HRP conjugate against Human WIF-1 with preservatives;	**508-01-03**	**1 vial**
Positive Control – one vial of Human WIF-1, lyophilized	**508-01-04**	**1 vial**
Sample Buffer – 3.5mL/bottle of buffered solution	**DB00**	**1 bottle**
Dilution Buffer- 60mL/bottle of buffered protein based solution with preservatives	**DB08**	**1 bottle**
Streptavidin-HRP Conjugate – 60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Wash Buffer – 50 ml/bottle, 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution- 11 ml/bottle of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution - 11 ml/bottle 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 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate as well as Dilution Buffer should be stored at -20°C 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 200-fold Concentrate 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 and seal 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.

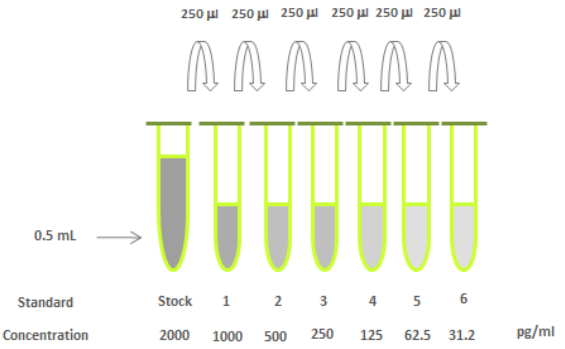
REAGENT PREPARATION

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.

WIF-1 Standard - Refer to vial label for reconstitution volume. Reconstitute the **WIF-1** Standard with 0.5 ml of Dilution Buffer. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of Dilution Buffer into the tube #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 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	500 µl	2000 pg/ml
# 1	250 µl of stock	250 µl	1000 pg/ml
# 2	250 µl of 1	250 µl	500 pg/ml
# 3	250 µl of 2	250 µl	250 pg/ml
# 4	250 µl of 3	250 µl	125 pg/ml
# 5	250 µl of 4	250 µl	62.5 pg/ml
# 6	250 µl of 5	250 µl	31.25 pg/ml



Detection Antibody- Reconstitute the **Detection Antibody Concentrate** with 105 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 105 µl of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 60 µl of 200-fold concentrated stock solution 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 0.5 mL of Dilution Buffer to prepare Positive

Control working solution. Allow it to sit for a minimum of 15 minutes with gentle agitation. *Note: Positive should be prepared and used immediately.*

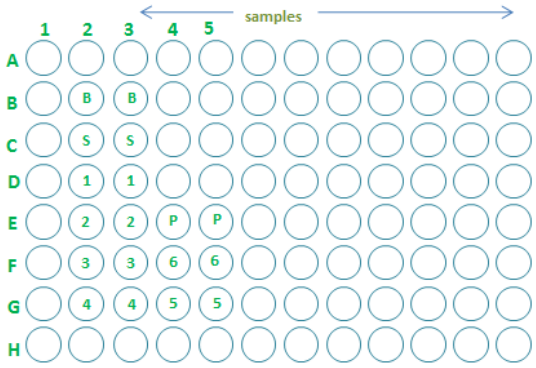
ASSAY PROCEDURE

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 and seal.
3. Add 30uL of **Sample Buffer** to all wells (Blank, Standard, Control and Samples)

3a. Add 100 µL of **Dilution Buffer** to Blank wells (B2, B3).

4. Add 100 µL of **Standard** (C2, C3 to G2, G3 and F4, F5 to G4, G5), **sample, or positive control** (E4, E5) per well. Cover with plate 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 plate 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 15-25 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 WIF-1 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.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed Human WIF-1.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of WIF-1 was 10 pg/mL.

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)	O.D. AT 450NM CORRECTED*
Blank	0 (0.099)
31.25	0.014
62.5	0.031
125	0.050
250	0.104
500	0.227
1000	0.504
2000	1.313

- *Lot No.: 20110470
- **Positive Control: 100 – 400 pg/mL

SPECIFICITY

This assay recognizes natural serum Human WIF-1. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/mL in a mid-range Human WIF-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY(%)
Human WIF-1	100
Mouse WIF-1	1.1
Human Wnt-7a	0
Human NOV	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 30 µl of Sample Buffer to all wells used
Add 100 µl 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 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 15-25 min on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min