

## HUMAN GALECTIN-1 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION  
OF HUMAN GALECTIN-1 CONCENTRATIONS  
IN SERUM, PLASMA AND MILK



THIS IS PROVIDED FOR DEMONSTRATION  
ONLY. ALWAYS REFER TO LOT SPECIFIC  
PROTOCOL 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:

**THIS KIT IS FOR ONE TIME USE ONLY.**

ELISA NAME	HUMAN GALECTIN-1 ELISA
Catalog No.	SK00198-06
Lot No.	
Formulation	96 T
Standard range	312 – 20,000 pg/mL
Sensitivity	100 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma, milk
Dilution Factor	<b>Optimal dilutions should be determined by each laboratory for each application</b>
Specificity	Human Galectin-1
Calibration	Human Galectin-1 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8° C
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

### ORDER CONTACT:

AVISCERA BIOSCIENCE, INC.  
2348 WALSH AVE., SUITE C  
SANTA CLARA, CA 95051  
USA

Tel: (408) 982 0300

Fax: (408) 982 0301

Email: [Sales@AvisceraBioscience.com](mailto:Sales@AvisceraBioscience.com)

Website: [www.AvisceraBioscience.com](http://www.AvisceraBioscience.com)

**DESCRIPTION**

This Human Galectin-1 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Galectin-3 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Galectin-1 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Galectin-1 samples.

**ASSAY OVERVIEW**

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human Galectin-1. The capture antibody can bind to the human Galectin-1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human Galectin-1 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 (TMB) is added to the wells and color develops in direct proportion to the amount of human Galectin-1 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.

\_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
<b>Galectin-3 Microplate</b> - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Galectin-1.	<b>198-06-01</b>	<b>1 plate</b>
<b>Galectin-1 Standard</b> – lot specific of recombinant human Galectin-1 in a buffered protein base with preservative; lyophilized.	<b>198-06-02</b>	<b>1 vial</b>
<b>Detection Antibody Concentrate</b> – lot specific, 10-fold concentrate of biotinylated antibody against Galectin-1 with preservative; lyophilized.	<b>198-06-03</b>	<b>1 vial</b>
<b>Positive Control</b> - one vial of recombinant human Galectin-1 with preservative; lyophilized.	<b>198-06-04</b>	<b>1 vial</b>
<b>Streptavidin-HRP Conjugate</b> - 120 µL/vial, 100-fold concentrated solution of Streptavidin-HRP conjugate.	<b>SAHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> - 30 mL of buffered protein based solution with preservative.	<b>DB10</b>	<b>1 bottle</b>
<b>HRP Diluent Solution</b> - 12 mL of buffered protein based solution with preservative.	<b>DB68C</b>	<b>1 bottle</b>
<b>Wash Buffer</b> - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	<b>WB01</b>	<b>1 bottle</b>
<b>TMB Substrate Solution</b> - 11 mL of TMB substrate solution.	<b>TMB01</b>	<b>1 bottle</b>
<b>Stop Solution</b> - 11 mL of 0.5M HCl.	<b>S-STOP</b>	<b>1 bottle</b>
<b>Plate Sealer</b>	<b>EAPS</b>	<b>1</b>
<b>Plastic Pouch</b>	<b>P01</b>	<b>1</b>

**STORAGE**

**Unopened Kit:** Store at 2 – 8° C for up to 1 months. For longer storage up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution buffer and HRP Diluent Solution should be stored at -20° C or -70° C. 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.

**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  $\leq -20^{\circ}\text{C}$ . 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  $\leq -20^{\circ}\text{C}$ . 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**

**Optimal dilutions should be determined by each laboratory for each application.**  
**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.

**Galectin-1 Standard** - Reconstitute the Galectin-1 standard with lot specific of Dilution Buffer. Pipette 250  $\mu\text{L}$  of Dilution Buffer into tubes #2 to #6. Use the high standard of **20,000 pg/mL** to produce a dilution series. Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	Lot specific	20 ng/ml
# 1	250 $\mu\text{L}$ of stock	250 $\mu\text{L}$	10 ng/ml
# 2	250 $\mu\text{L}$ of 1	250 $\mu\text{L}$	5 ng/ml
# 3	250 $\mu\text{L}$ of 2	250 $\mu\text{L}$	2.5 ng/ml
# 4	250 $\mu\text{L}$ of 3	250 $\mu\text{L}$	1.25 ng/ml
# 5	250 $\mu\text{L}$ of 4	250 $\mu\text{L}$	0.625 ng/ml
# 6	250 $\mu\text{L}$ of 5	250 $\mu\text{L}$	0.3125 ng/ml

**Positive Control** - Reconstitute the Positive Control with lot specific of Dilution Buffer.

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

**Streptavidin-HRP Conjugate** - Transfer 120  $\mu\text{L}$  of 100-fold concentrated stock solution to 11.88 mL of **HRP Diluent Solution (DB68-C)** to prepare working solution. (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. Add 100  $\mu\text{L}$  per well of **Dilution Buffer** to Blank wells.
3. Add 100  $\mu\text{L}$  of **Standard dilutions, samples, or positive control** per well. Cover with plate sealer.

Incubate for 2 hours on plate shaker at room temperature.

4. 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  $\mu$ L) using a squirt bottle, manifold dispenser, or auto-washer. 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.
5. Add 100  $\mu$ L of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on plate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100  $\mu$ L of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on plate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100  $\mu$ L of **Substrate Solution** to each well. Incubate for lot specific on a plate shaker at room temperature. **Protect from light.**
10. Add 100  $\mu$ L 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.
11. Determine the optical density of each well using a microplate 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 Galectin-3 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 STANDARD CURVE

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









STANDARD (NG/ML)	CORRECTED (450NM)
Blank	0 (lot specific)
0.312	0.044
0.625	0.083
1.25	0.157
2.5	0.310
5	0.622
10	1.126
20	2.125

### SPECIFICITY

PROTEINS	CROSSREACTIVITY (%)
Human Galectin-1	100
Human Galectin-3	0

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**SUMMARY OF ASSAY PROCEDURE**

<b>PREPARE REAGENTS, SAMPLES AND STANDARDS</b>

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

Aspirate and wash 4 times.

Add 100 µL Detection Antibody working solution to each well. Incubate for 2 hours on 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. <b>Protect from light.</b>

Aspirate and wash 4 times.

Add 100 µL Substrate Solution to each well. Incubate refer to lot specific on the plate shaker at RT. <b>Protect from light.</b>

Add 100 µL Stop Solution to each well. Read at 450nm.