

## HUMAN CHITINASE-3-LIKE 1 (YKL-40) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION  
OF HUMAN CHITINASE-3-LIKE 1 (YKL-40)  
CONCENTRATIONS IN CELL CULTURE  
SUPERNATES, SERUM, AND PLASMA



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

### PURCHASE INFORMATION:

ELISA NAME	HUMAN YKL-40 ELISA
Catalog No.	SK00088-02
Lot No.	
Formulation	96 T
Standard range	0.25 – 16 ng/mL
Sensitivity	0.125 ng/mL
Sample Volume	100 µl
Dilution Factor	200 (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human YKL-40 only
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2°C - 8°C

Order Contact:  
**AVISCIERA BIOSCIENCE, INC.**  
 2348 Walsh Ave., Suite C  
 Santa Clara, CA 95051  
 Tel: (408) 982 0300  
 Fax: (408) 982 0301  
 Email: [Info@AvisceraBioscience.com](mailto:Info@AvisceraBioscience.com)  
[www.AvisceraBioscience.com](http://www.AvisceraBioscience.com)

## INTRODUCTION

Human Chitinase-3-like 1 (YKL-40) immunoassay is a solid phase ELISA designed to measure human YKL-40 in cell culture supernatants, serum, and plasma. It contains recombinant human YKL-40 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human YKL-40. Results obtained with naturally occurring YKL-40 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 YKL-40.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for YKL-40 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any YKL-40 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for YKL-40 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin 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 YKL-40 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
<b>YKL-40 Microplate</b> – 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human YKL-40.	<b>088-02-01</b>	<b>1 plate</b>
<b>YKL-40 Standard</b> – 8 ng/vial of recombinant human YKL-40 in a buffered protein base with preservatives; lyophilized.	<b>088-02-02</b>	<b>2 vials</b>
<b>Detection Antibody Concentrate</b> – 1.05mL/vial, 10-fold concentrate of biotinylated polyclonal antibody against human YKL-40 with preservatives; lyophilized.	<b>088-02-03</b>	<b>1 vial</b>
<b>Positive Control</b> – one vial of recombinant human YKL-40, lyophilized	<b>088-02-04</b>	<b>1 vial</b>
<b>Streptavidin-HRP Conjugate</b> – 120 uL/vial, 100-fold concentrated solution of Streptavidin conjugated to HRP with preservatives	<b>SAHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> – 60 mL of buffered protein based solution with preservatives	<b>DB01</b>	<b>2 bottles</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 piece</b>
<b>Plastic Pouch</b>	<b>P01</b>	<b>1 piece</b>

## 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 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 100-fold concentrate (protect from light) and other components may be stored at 2 - 8°C for up to 8 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 after opening.

**OTHER SUPPLIES REQUIRED**

- Microplate reader capable of measuring absorbance at 450 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.

**SAMPLE COLLECTION AND STORAGE**

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000x 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 1000x 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 need a 200-fold dilution. A suggested 200-fold dilution is 5 µL sample + 995 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application.**

**Use polypropylene test tubes.**

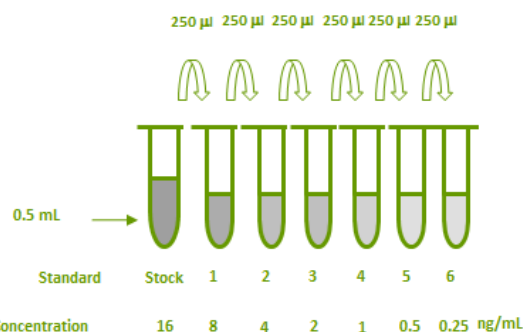
**REAGENT PREPARATION**

**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 Wash Buffer.

**YKL-40 Standard - Refer to vial label for reconstitution volume.** Reconstitute the YKL-40 standard with 0.5 mL of Dilution Buffer. This reconstitution produces a stock solution of 16 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 into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 16 ng/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	0.5 ml	16 ng/ml
# 1	250µl of stock	250µl	8 ng/ml
# 2	250µl of 1	250µl	4 ng/ml
# 3	250µl of 2	250µl	2 ng/ml
# 4	250µl of 3	250µl	1 ng/ml
# 5	250µl of 4	250µl	0.5 ng/ml
# 6	250µl of 5	250µl	0.25 ng/ml



**Positive Control** – Reconstitute the **Positive Control** with 0.5 mL Dilution Buffer. **Note:** Positive Control should be prepared and used within a few days if store at -20°C or -70°C.

**Detection Antibody Concentrate** – Reconstitute the Detection Antibody Concentrate with 1.05mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05mL of

10-fold concentrated stock solution to prepare working solution.

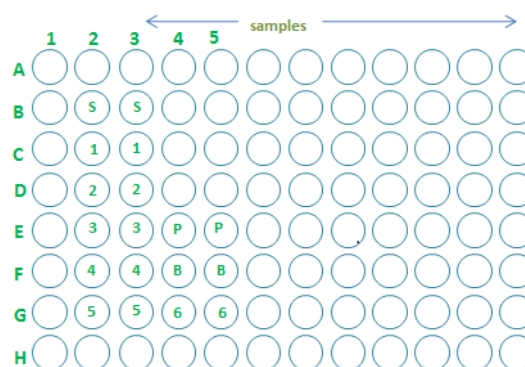
**Streptavidin-HRP Conjugate** - Pipette 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120  $\mu$ L of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP conjugate should be used within a few days (protect from light).

### 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 microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100  $\mu$ L of **Dilution Buffer** to Blank wells (F4, F5).
4. Add 100  $\mu$ L of **Standard** (B2, B3 to G2, G3 and G4, G5), **sample**, or **positive control** (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on 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 **1x Wash Buffer** (300  $\mu$ L) 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  $\mu$ L of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100  $\mu$ L 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  $\mu$ L of **Substrate Solution** to each well. Incubate for 6-10 minutes on micro-plate shaker at room temperature. **Protect from light.**

11. 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.
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, 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 YKL-40 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 recombinant human YKL-40.

**SENSITIVITY**

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of YKL-40 was 0.125 ng/mL.

**TYPICAL DATA**

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

YKL-40 (ng/mL)	Average OD450 (Corrected)*
Blank	0 (0.0106)
0.25	0.021
0.5	0.039
1	0.083
2	0.133
4	0.269
8	0.570
16	1.254

\*Lot No.:

\*\* Positive Control:

**SPECIFICITY**

	Cross-reactivity
Human Chitinase-3-like 1	100%
Human Chitinase-3-like 2	0
Human Chitotriosidase	0
Mouse Chitinase-3-like 1	0

**REFERENCES:**

1. Masajtis-Zagajewska A, et al. Effect of mononidine and amlodipine on serum YKL-40, plasma lipids and insulin sensitivity in insulin-resistant hypertensive patients – a randomized, crossover trial. *Hypertens Res.* 2010 Apr;33(4):348-53. Epub 2010 Feb 5.
2. Furuhashi K, et al. Increased expression of YKL-40, a chitinase-like protein, in serum and lung of patients with idiopathic pulmonary fibrosis. *Respir Med.* 2010 Mar 26. [Epub ahead of print]
3. Comabella M, et al. Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. *Brain.* 2010 Apr;133(Pt 4):1082-93. Epub 2010 Mar 17.
4. Chupp GL, et al. A chitinase-like protein in the lung and circulation of patients with

severe asthma. *N Engl J Med.* 2007 Nov 15;357(20):2016-27

5. Ober C, et al. Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. *N Engl J Med.* 2008 Apr 17;358(16):1682-91. Epub 2008 Apr.

**SUMMARY OF ASSAY PROCEDURE**

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of standard, samples, positive control to the 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 plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Streptavidin-HRP Conjugate working solution to each well. Incubate 60 min on plate shaker at RT. <b>Protect from light.</b>
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 6-10 min on plate shaker. <b>Protect from light.</b>
↓
Add 100 µL Stop Solution to each well. Read 450nm within 15 min