

An enzyme immunoassay for the quantitation of YKL-40 in serum and other biological fluids

For Research Use Only

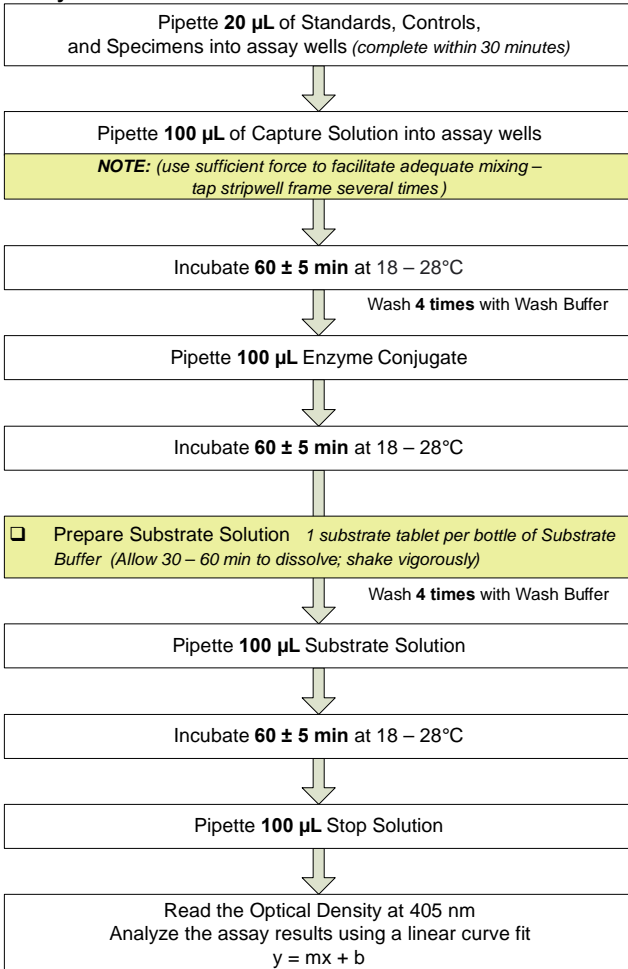
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MicroVue™ YKL-40 EIA Summary

Reagent Preparation

- Dilute 10X Wash Buffer 1:10 with DI Water
- Prepare each vial of Enzyme Conjugate with 7 mL of Reconstitution Buffer (Use within 24 hours)

Assay Procedure



SUMMARY AND EXPLANATION

YKL-40, a member of the mammalian chitinase like protein class, is a 40 kDA heparin binding glycoprotein.¹⁻³ It shares amino acid sequence homology to non-mammalian chitinases but demonstrates no chitinase activity.^{1,4} The name YKL-40 is derived from the protein's molecular weight and three N-terminus amino acids (tyrosine, lysine and leucine).⁵ The gene for YKL-40 has been identified (CHI3L1, located on chromosome 1q31-q32),⁶ and the protein's structure described,⁷ but the protein's site and mode of binding to cell surface receptors remains unknown.

The biological function of YKL-40 remains largely unknown and is a field of extensive scientific debate. YKL-40 has been shown to be a potent growth factor for connective tissue cells⁸⁻⁹ and a potent migration factor for endothelial cells.¹⁰ Several research studies have demonstrated substantial levels of YKL-40 in environments with inflammation or where remodeling of the extracellular matrix (ECM) occurs,¹⁶⁻²³ including various cancers, active rheumatoid arthritis, inflammatory bowel diseases, severe bacterial infections, and liver fibrosis.

PRINCIPLE OF THE PROCEDURE

The MicroVue YKL-40 enzyme immunoassay kit for the detection of YKL-40 in experimental samples is a three-step procedure utilizing (1) a microassay plate coated with streptavidin and a biotinylated murine monoclonal antibody to human YKL-40, (2) an AP-conjugated rabbit polyclonal antibody to YKL-40, and (3) a chromogenic substrate.

In the first step, Standards, Controls, and test specimens are added to the avidin coated assay wells along with the capture solution containing a biotinylated F(ab) fragment of a murine monoclonal antibody to YKL-40. The monoclonal antibody binds to YKL-40 in the Standards, Controls, or specimens, and the biotin binds to the avidin on the microwell plate, immobilizing the antibody. After an incubation period, a wash cycle removes any unbound material.

In the second step, alkaline phosphatase (AP) conjugated rabbit anti-YKL-40 is added to each assay well. The enzyme conjugated anti-YKL-40 binds to the immobilized YKL-40 captured in the first step. After an incubation period, a wash cycle removes any unbound conjugate.

In the third step, p-nitrophenyl phosphate (pNPP), a chromogenic substrate solution, is added to the assay wells. The bound AP reacts with the substrate, forming a yellow color. After an incubation period, the reaction is stopped chemically, and the color intensity is measured spectrophotometrically at A₄₀₅. The color intensity of the reaction mixture is proportional to the concentration of YKL-40 present in the test specimens, Standards and Controls. Results are calculated from the generated standard curve using linear regression analysis.

REAGENTS AND MATERIALS PROVIDED

96 Assays for YKL-40

MicroVue YKL-40 EIA Kit contains the following:

- A** YKL-40 Standards: Parts 4620-4625 A, 1.4 mL
- B** YKL-40 Standards: Parts 4620-4625 B - F, 0.3 mL each
- C** YKL-40 Standards: Parts 4620-4625 C (A = 0, B = 20, C = 50, D = 100, E = 200, F = 300 ng/mL)
- D** YKL-40 purified from osteosarcoma MG-63 cells in a buffered solution with stabilizer and sodium azide (0.1%) as a preservative
- E** YKL-40 purified from osteosarcoma MG-63 cells in a buffered solution with stabilizer and sodium azide (0.1%) as a preservative
- F** YKL-40 purified from osteosarcoma MG-63 cells in a buffered solution with stabilizer and sodium azide (0.1%) as a preservative

- L Low/High Controls** **Parts 4626, 4627** **1 each**
H YKL-40 purified from osteosarcoma MG-63 cells in a buffered solution with stabilizer and sodium azide (0.1%) as a preservative
- 1 Coated Strips** **Part 4634** **12 each**
Streptavidin adsorbed onto 12 eight-well breakaway strips in a resealable foil pouch
- 2 Stop Solution** **Part 4702** **15 mL**
0.5N NaOH
- 3 10X Wash Buffer** **Part 4703** **55 mL**
Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative
- 4 Reconstitution Buffer** **Part 4628** **27 mL**
Nonionic detergent in a buffered solution containing food dye, stabilizers, and sodium azide (0.1%) as a preservative
- 5 Substrate Buffer** **Part 4705** **3 x 10 mL**
A diethanolamine and magnesium chloride solution containing sodium azide (0.05%) as a preservative
- 6 Substrate Tablets** **Part 0012** **3 x 20 mg**
p-Nitrophenyl phosphate
- 7 Enzyme Conjugate** **Part 4633** **3 each**
Lyophilized rabbit polyclonal anti-YKL-40 antibody conjugated to alkaline phosphatase containing buffer salts and stabilizers
- 8 Capture Solution** **Part 4629** **12 mL**
Mouse monoclonal anti-YKL-40 F(ab) antibody conjugated to biotin in a buffered solution containing food dye, stabilizers, and sodium azide (0.1%) as a preservative

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes to deliver 25-300 µL and 500 µL
- Multichannel pipettes
- Items suitable for liquid measurement of 7-600 mL
- Container for wash buffer dilution
- Deionized or distilled water
- Plate reader capable of reading at 405 nm
- Linear curve fitting software

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for Use in Diagnostic Procedures.
2. Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.²⁴
3. Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
4. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5. Wear suitable protective clothing, gloves, and eye/face protection when handling contents of this kit.
6. Store assay reagents as indicated.
7. Do not use Coated Strips if pouch is punctured.
8. Test each sample in duplicate.
9. 0.5N NaOH is considered corrosive and can cause irritation. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.

10. Sodium azide is used as a preservative. Incidental contact with or ingestion of buffers containing sodium azide may cause irritation to the skin, eyes, or mouth. Only use buffers for intended purposes and avoid contact with acids. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build-up.
11. The substrate buffer contains diethanolamine and may cause irritation to the eyes and/or skin with prolonged contact. Contacted areas should be immediately washed with soap and water.
12. Use of multichannel pipets or repeat pipettors is recommended to ensure timely delivery of reagents.
13. For accurate measurement of samples, add samples and standards precisely. Pipet carefully using only calibrated equipment.
14. Proper collection and storage of test specimens are essential for accurate results (see *SPECIMEN COLLECTION AND STORAGE*).
15. Avoid microbial or cross-contamination of specimens or reagents.
16. Do not use a microassay well for more than one test.
17. Decontaminate all specimens, reagents, and materials by soaking for a minimum of 30 minutes in a 1:10 solution of household bleach (sodium hypochlorite) or autoclave at 121°C for 30 minutes at 15 psi.
18. Using incubation times and temperatures other than those indicated in the *ASSAY PROCEDURE* section may give erroneous results.
19. Do not allow microassay wells to dry once the assay has begun.
20. When removing liquid from the microassay wells, do not scrape or touch the bottom of the wells.
21. Hyperlipemic or contaminated specimens may give erroneous results.
22. To avoid aerosol formation during washing, use an apparatus to aspirate the wash fluid into a bottle containing household bleach.
23. A wash bottle or automated filling device should be used to wash the plate (*ASSAY PROCEDURE*, Step 6). For best results, do not use a multichannel pipette to wash the microassay plate.

REAGENT PREPARATION

All reagents should be equilibrated to 18-28°C prior to use.

Determine amount of each reagent required for the number of strips to be used.

# of Strips	4	6	8	12
# of Samples (tested in duplicate)	8	16	24	40
Enzyme Conjugate (vial)	1	1	2*	2*
Substrate Buffer (bottle)	1	1	2*	2*
1X Wash Buffer (mL)	100	150	200	300

*When more than one bottle or vial is to be used, combine the contents and mix prior to use.

Coated Strips

Remove Stripwell Frame and the required number of Coated Strips from the pouch (see table in Assay Procedure section). Ensure that the pouch containing any unused strips is completely resealed and contains desiccant.

Wash Buffer

Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer 1:10 with deionized water. Store at 18-28°C. Use 1X Wash Buffer within 4 weeks of preparation.

Enzyme Conjugate

Prepare Enzyme Conjugate within 24 hours of use. Reconstitute each required vial of Enzyme Conjugate (see table) with 7 mL of Reconstitution Buffer. Store reconstituted Enzyme Conjugate at 18-28°C until use. Discard remaining Enzyme Conjugate after use.

Working Substrate Solution

The Substrate Buffer must be brought to 18-28°C before beginning the assay (two hours to overnight recommended). Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of room temperature Substrate Buffer (see table). Allow 30-60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.

STORAGE

Store the kit at 2-8°C. Store unused reagents at 2-8°C. Store 1X Wash Buffer (10X diluted) at 18-28°C.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Cloudiness, discoloration, or offensive odor may indicate instability or deterioration of kit reagents. If this occurs, discard the reagent.

SPECIMEN COLLECTION AND STORAGE

Handle and dispose of all specimens using Universal Precautions.

The MicroVue YKL-40 assay kit has been validated for use with human serum and EDTA plasma specimens by Quidel Corporation. Additionally, human cerebrospinal fluid,¹² BAL fluid,¹³ synovial fluid,¹⁴ as well as human cell culture supernatants,¹⁵ have been tested and shown to be suitable for use in the assay in independent studies.

Serum or plasma should be collected using standard venipuncture techniques. Care should be taken in the handling of specimens to avoid hemolysis. Specimens should be processed from whole blood as quickly as possible. When left at room temperature, serum specimens are stable for up to 3 hours. Plasma specimens are stable for up to eight hours at room temperature. If blood samples cannot be processed in this time frame, specimens may be stored at 4°C until processed. Serum and plasma are stable for up to 7 days at 2-8°C. Quidel has confirmed that serum and plasma specimens may be stored at or below -20°C storage for samples up to eight years.

ASSAY PROCEDURE

Read entire product insert before beginning the assay. See **WARNINGS AND PRECAUTIONS** and **REAGENT PREPARATION**.

Sample / Capture Solution Incubation

1. Allow pouch of Coated Strips to equilibrate to 18-28°C before opening. Remove Stripwell Frame and the required number of Coated Strips from the pouch (see table in **REAGENT PREPARATION**). Ensure that the pouch containing unused strips is completely resealed and contains desiccant.
2. Place desired number of Coated Strips in Stripwell Frame. Label strips for orientation in case of accidental removal from Stripwell Frame.
3. Add 20 µL Standard, Control, or sample to each well of the Coated Strips. This step should be completed within 30 minutes.
4. Add 100 µL of Capture Solution to each well. Dispense Capture Solution with sufficient force to ensure adequate mixing. Tap Stripwell Frame several times. **NOTE:** *Adequate mixing of Standards, Controls, and samples with Capture Solution is required for acceptable assay performance.*
5. Incubate 60 ± 5 minutes at 18-28°C.

Enzyme Conjugate Incubation

6. Manually invert/empty strips. Add at least 250 µL of 1X Wash Buffer (refer to **REAGENT PREPARATION**) to each well and manually invert/empty strips. Repeat three more times for a total of four washes. Vigorously blot the strips dry on paper towels after the last wash. See **WARNINGS AND PRECAUTIONS**, item 23 for additional information regarding washing.
7. Add 100 µL of reconstituted Enzyme Conjugate (refer to **REAGENT PREPARATION**) to each well. Discard remaining reconstituted Enzyme Conjugate after use.
8. Incubate 60 ± 5 minutes at 18-28°C.

Substrate Incubation

9. Repeat wash as indicated in step 6.
10. Add 100 µL of Working Substrate Solution (refer to **REAGENT PREPARATION**) to each well.
11. Incubate for 60 ± 5 minutes at 18-28°C. **NOTE:** *If room temperature cannot be maintained between 18-28°C, monitor the development of signal in the YKL-40 Standard F wells; stop the reaction when the optical density reaches at least 1.2; then read the strip(s).*

Stop/Read

12. Add 100 µL of Stop Solution to each well. Add Stop Solution in the same pattern and time intervals as the Working Substrate Solution addition.
13. Read the optical density at 405 nm. Assure that no large bubbles are present in wells and that the bottom of the strips are clean. Strips should be read within **15 minutes** of Stop Solution addition. **NOTE:** *If the plate reader is incapable of linear optical density readings between 2.0 and 3.0, monitor the development of substrate in the YKL-40 Standard F wells; stop the reaction when the optical density reaches at least 1.2 but less than 2.0; then read the strip(s).*

14. Calculate the results as described in the *INTERPRETATION OF RESULTS* section of this document.

QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Quidel Corporation. The optical density values are provided but should be used as a guideline only. The results obtained by your laboratory may differ.

Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable and the samples should be repeated.

Dilute samples greater than 300 ng/mL in Standard A and retest. Be sure to include the dilution factor in the final calculation. If the optical density of the YKL-40 Standard A is greater than 0.4, the results should be considered questionable and the samples should be repeated.

INTERPRETATION OF RESULTS

The standard curve for the YKL-40 EIA Kit is generated using the A_{405} values for each Standard (on the Y axis) and the assigned concentration for each YKL-40 Standard (on the X axis). Most computers and calculators are capable of performing these calculations.

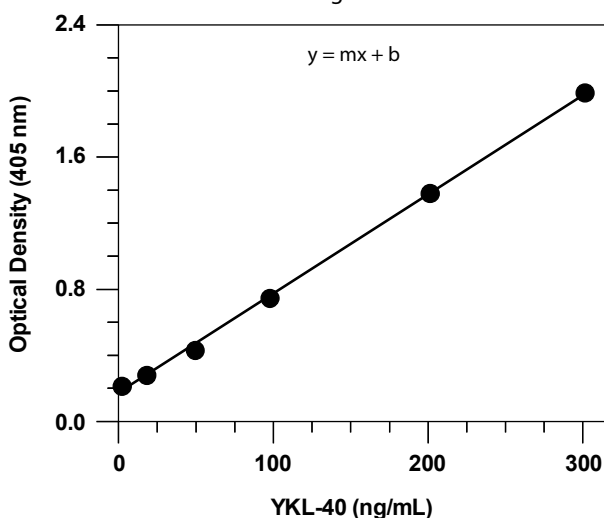
Alternatively, the data may be graphed manually and the values (ng/mL) of the test specimens read directly from the best-fit line of the Standard Curve.

Representative Standard Curve

An example of a typical standard curve is shown in Figure 1 below.

Standard YKL-40 levels: 0, 20, 50, 100, 200, 300 ng/mL

Figure 1



EXAMPLE VALUES

Quidel tested EDTA plasma (102) and sera (329) from apparently normal adult donors ≤ 60 years of age using the MicroVue YKL-40 assay kit. Results are provided in the table below.

	n	Median	Range
EDTA Plasma	102	33	20-130 ng/mL
Serum (female)	226	41.15	25-93 ng/mL
Serum (male)	103	45.60	24-125 ng/mL

Each laboratory should establish its own reference range.

PERFORMANCE OF THE TEST

Limits of Detection

LOD: The Limit of Detection (LOD) for the MicroVue YKL-40 EIA Kit is 5.4 ng/mL as determined by the upper 3SD limit in a zero standard study.

ULOQ: The Upper Limit of Quantitation (ULOQ) for the MicroVue YKL-40 EIA is 300ng/mL as determined by the highest concentration of highly purified YKL-40 that met NCCLS criteria for accuracy and precision. Dilution of elevated specimens would increase ULOQ.

LLOQ: The Lower Limit of Quantitation (LLOQ) for the MicroVue YKL-40 EIA is 15.6 ng/mL as determined by the lowest concentration of YKL-40 in a serum specimen that met NCCLS criteria for accuracy and precision upon dilution.

Recovery - Spike Recovery

Spike recovery was determined by adding a known quantity of purified YKL-40 to serum samples with different levels of endogenous YKL-40. Typical results are provided below

Sample	Endogenous (ng/mL)	Added (ng/mL)	Observed (ng/mL)	Recovery (%)
1	27.6	34.4	60.2	95
2	139.1	34.4	172.5	97
3	170.4	34.4	206.9	106

Recovery - Linearity

Linearity was determined by diluting samples in Standard A and comparing observed values with expected values. Typical results are provided below.

Sample	Dilution Factor	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
1	neat	217.8	-	-
	1:2	103.1	108.9	95
	1:4	50.8	54.5	93
	1:8	26.2	27.2	96
2	neat	147.2	-	-
	1:2	70.5	73.6	96
	1:4	37.5	36.8	102
3	neat	84.5	-	-
	1:2	40.7	42.2	96
	1:4	23.0	21.1	109

Precision

Within-run and between-run precision were determined by assaying up to 22 serum samples in 6 different runs.

Sample	YKL-40 (ng/mL)	Within-run ¹ CV%	Between-run ² CV%
1	51.8	6.6	6.8
2	177.8	5.6	7.0
3	262.9	5.8	6.0

¹ n = 21 or 22 replicates

² n = 6 runs

Interfering Substances

The following substances were tested at the specified concentrations, and were found not to interfere with the assay.

Substance	Concentration
Hemoglobin	500 mg/dL
Bilirubin	30 mg/dL
Triglycerides	3000 mg/dL
Total Protein	3 – 12 g/dL

Cross-Reactivity

The MicroVue YKL-40 assay has been used to measure YKL-40 levels in sera and plasma from cynomolgus macaques, rhesus monkeys and baboons. Further species cross-reactivity has not been confirmed by Quidel.

Drug Interferences

Various concentrations of drugs were added to serum specimens. The following drugs were found not to interfere at the highest concentrations shown:

Substance	Highest Concentration
Aspirin	5 mg/mL
Azathioprine	0.5 mg/mL
D-penicillamine	1 mg/mL
Dexamethasone	1 mg/mL
Diclofenac	5 mg/mL
Etodolac	10 mg/mL
Fenbufen	0.1 mg/mL
Fenoprofen	5 mg/mL
Flurbiprofen	0.05 mg/mL
Gold thioglucose	10 mg/mL
Hydroxychloroquine	5 mg/mL
Ibuprofen	10 mg/mL
Indomethacin	10 mg/mL
Ketoprofen	1 mg/mL
Meclofenamate	1 mg/mL
Mefenamic acid	0.1 mg/mL
Methotrexate	10 mg/mL
Nabumetone	0.1 mg/mL
Naproxen	5 mg/mL
Piroxicam	5 mg/mL
Prednisone	1 mg/mL
Sulfasalazine	1 mg/mL
Sulindac	5 mg/mL
Tolmetin	10 mg/mL

ASSISTANCE

To place an order or for technical assistance, please contact a Quidel Representative at 800-524-6318 or 408-616-4301, Monday through Friday, between 8:00 a.m. and 5:00 p.m., Pacific Time. Orders may also be placed by fax at 408-616-4310.

For services outside the U.S., please contact your local distributor. Additional information about Quidel, as well as Quidel's products and distributors, can be found on our website at www.quidel.com.

Covered by U.S. Patent Nos. 5,935,798; 6,579,684; 6,794,150; 7,230,086; other patents pending.

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GLOSSARY



Catalog Number



Consult Instructions for Use



Contains sufficient for <n> tests



Instructions for Use on CDROM



Manufacturer



Contents / Contains



Biological Risks



Temperature Limitation

REF 8020 – MICROVUE[®] YKL-40 EIA Kit
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