

METRA™ YKL-40 EIA kit

96 Assays for YKL-40 in Serum

For Research Use Only

Store at 2-8°C

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Made in USA

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QUIDEL®

Read the entire product insert thoroughly before beginning the assay. The Metra™ YKL-40 kit should be stored at 2-8°C until use.

QUICK GUIDE TO ASSAY STEPS

1. Add 20 µL Standards, Controls, and samples.
2. Add 100 µL Capture Solution.
3. Incubate 60 ± 5 minutes at room temperature.
4. Wash 4 times with 1X Wash Buffer.
5. Add 100 µL Enzyme Conjugate.
6. Incubate 60 ± 5 minutes at room temperature.
7. Wash 4 times with 1X Wash Buffer.
8. Add 100 µL Working Substrate Solution.
9. Incubate 60 ± 5 minutes at room temperature.
10. Add 100 µL Stop Solution and read OD at 405 nm.

SUMMARY AND EXPLANATION

YKL-40, also known as human cartilage glycoprotein 39 (HC gp-39), is a 40 kilodalton glycoprotein first described in whey secretions of nonlactating cows¹. Subsequently, its production by chondrocytes, synovial cells, activated macrophages, neutrophils, and osteosarcoma cells (MG-63) has been reported²⁻⁶. Though the role of YKL-40 is unknown at present, its pattern of expression and observed associations with various disease activities suggest it plays a role in tissue remodeling. It has both heparin and chitin binding domains and appears to be an autoantigen in rheumatoid arthritis^{3,4,7}.

The first method for the measure of YKL-40 in serum (and synovial fluid) - an RIA - was described by Johansen et al in 1993⁸. They have used the RIA in a number of clinical investigations describing its possible utility as a marker of joint destruction activity in rheumatoid arthritis and osteoarthritis, liver fibrosis in alcoholic cirrhosis, and survival following recurring breast cancer or colorectal cancer⁸⁻¹².

PRINCIPLE OF THE PROCEDURE

The YKL-40 assay is a sandwich enzyme immunoassay in a microtiter stripwell format. The Fab fragment of a monoclonal anti-YKL-40 antibody conjugated to biotin binds to streptavidin on the strip and captures YKL-40 in a standard, control, or sample. A polyclonal anti-YKL-40 antibody conjugated to alkaline phosphatase binds to the captured YKL-40. Bound enzyme activity is detected with p-nitrophenyl phosphate as substrate.

REAGENTS AND MATERIALS

Metra YKL-40 part number 8020 contains the following:

Substrate Tablets	Part 0012	3 each
p-Nitrophenyl phosphate (20mg each).		
YKL-40 Standards:	Parts 4620 - 4625	0.3 mL each
0, 20, 50, 100, 200, 300 ng/mL		
YKL-40 purified from osteosarcoma MG-63 cells in a buffered solution containing stabilizer and sodium azide (0.1%) as a preservative.		
Low/High Controls	Parts 4626, 4627	0.3 mL each
YKL-40 purified from osteosarcoma MG-63 cells in a buffered solution containing stabilizer and sodium azide (0.1%) as a preservative.		
Reconstitution Buffer	Part 4628	27 mL
Nonionic detergent in a buffered solution containing food dye, stabilizers, and sodium azide (0.1%) as a preservative.		
Capture Solution	Part 4629	12 mL
Mouse monoclonal anti-YKL-40 Fab antibody conjugated to biotin in a buffered solution containing food dye, stabilizers, and sodium azide (0.1%) as a preservative.		
Enzyme Conjugate	Part 4633	3 each
Lyophilized rabbit polyclonal anti-YKL-40 antibody conjugated to alkaline phosphatase containing buffer salts and stabilizers.		
Coated Strips, 1 X 8 wells	Part 4634	1 each
Streptavidin adsorbed onto stripwells.		
Stop Solution	Part 4702	15 mL
1N NaOH		
10X Wash Buffer	Part 4703	55 mL
Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative.		
Substrate Buffer	Part 4705	10 mL
A diethanolamine and magnesium chloride solution containing sodium azide (0.05%) as a preservative.		

WARNINGS

1. All serum samples should be treated as potentially biohazardous material.
2. 1N NaOH is poisonous and can cause severe burns. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
3. Sodium azide is used as a preservative. It may be fatal if swallowed or adsorbed through the skin. Do not mix 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.
4. Test kits and components should be disposed of in a manner consistent with relevant regulations.

PRECAUTIONS

1. All reagents supplied should be used as an integral unit prior to the expiration date indicated on the package label.
2. Assay reagents should be stored as indicated.
3. Do not use Coated Strips if pouch is punctured.
4. Samples greater than 300 ng/mL should be further diluted in Standard A and retested. Be sure to include the dilution factor in the final calculation.
5. Each sample should be tested in duplicate.
6. A standard curve must be performed with each assay.
7. A linear calibration curve fit must be used for accurate results. Equation: $y = mx + b$
8. This assay may be performed with any validated washing method.
9. 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 OD values provided are to 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.

10. If room temperature cannot be maintained between 18-28°C, monitor the development of substrate in the YKL-40 Standard F wells; stop the reaction when the OD reaches at least 1.2; then read the strip(s).
11. If the OD of the YKL-40 Standard A is greater than 0.4, the results should be considered questionable and the samples should be repeated.
12. If the plate reader is incapable of linear OD readings between 2.0 and 3.0, monitor the development of substrate in the YKL-40 Standard F wells; stop the reaction when the OD reaches at least 1.2 but less than 2.0; then read the strip(s).
13. Use of multichannel pipettes or repeat pipetors is recommended to ensure timely delivery of reagents.
14. For accurate measurement of samples, the addition of samples and standards must be precise. Pipet carefully using only calibrated equipment.
15. Adequate mixing of Standards, Controls and samples with Capture Solution is required for acceptable assay performance. Dispense Capture Solution forcefully into microtiter wells.

REAGENT PREPARATION AND STORAGE

Equilibrate reagents needed for each run to room temperature (18-28°C) prior to use. Store unused reagents at 2-8°C.

1. 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 unused strips is completely resealed and contains desiccant.
2. Wash Buffer
Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer 1:10 with deionized water. Store at room temperature (18-28°C). Use 1X Wash Buffer within 4 weeks.
3. 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 room temperature (18-28°C) until use. Discard remaining Enzyme Conjugate after use.
4. Working Substrate Solution
The Substrate Buffer must be brought to room temperature (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.

SPECIMEN COLLECTION AND STORAGE

Collect serum using standard venipuncture technique. Specimens should be collected without anticoagulants and processed to avoid hemolysis. Allow the blood to clot and separate the serum by centrifugation. Serum can be stored for 7 days at 2-8°C, or freeze the sample at $\leq -20^{\circ}\text{C}$ for longer storage. Do not subject the samples to more than 5 freeze/thaw cycles.

YKL-40 ASSAY PROCEDURE

Metra YKL-40 part number 8020

<u>Kit Contents</u>	<u>Qty/Vol</u>	<u>Part</u>
Substrate Tablets	3 each	0012
Standard A (YKL-40 0 ng/mL)	1.4 mL	4620
Standard B (YKL-40 20 ng/mL)	0.3 mL	4621
Standard C (YKL-40 50 ng/mL)	0.3 mL	4622
Standard D (YKL-40 100 ng/mL)	0.3 mL	4623
Standard E (YKL-40 200 ng/mL)	0.3 mL	4624
Standard F (YKL-40 300 ng/mL)	0.3 mL	4625
Control, Low	0.3 mL	4626
Control, High	0.3 mL	4627
Reconstitution Buffer	27 mL	4628
Capture Solution	12 mL	4629
Enzyme Conjugate (lyophilized)	3 each	4633
Coated Strips (12)	1 each	4634
Stop Solution	15 mL	4702
10X Wash Buffer	55 mL	4703
Substrate Buffer (3)	10 mL	4705

Materials Required BUT NOT Provided

Micropipettes to deliver 20, 100, and ≥ 250 μL
Multichannel pipettes
Labware 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 calibration curve fitting software

YKL-40 ASSAY PROCEDURE (CONT.)

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) †	150	200	250	300

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

† Automated plate washers may require larger volumes.

Sample/Capture Solution Incubation

1. Allow pouch of Coated Strips to equilibrate to room temperature before opening. Remove Stripwell Frame and the required number of Coated Strips from the pouch (see table). Ensure that the pouch containing unused strips is completely resealed and contains desiccant.
2. Place desired number of Coated Strips in Stripwell Frame just prior to use. Label strips to prevent mix-up in case of accidental removal from Stripwell Frame.
3. Add 20 μ L Standard, Control, or sample to each well of the Coated Strips. This step should be completed within 30 minutes.
4. Add 100 μ L of Capture Solution to each well. Dispense Capture Solution with sufficient force to ensure adequate mixing. Tap Stripwell Frame several times.
5. Incubate 60 ± 5 minutes at room temperature (18-28°C).

Enzyme Conjugate Incubation

1. Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer concentrate 1:10 with deionized water. Store at room temperature (18-28°C). Use 1X Wash Buffer within 4 weeks.
2. Manually invert/empty strips. Add at least 250 μ L of 1X Wash Buffer 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.
3. 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 room temperature (18-28°C) until use.
4. Add 100 μ L of reconstituted Enzyme Conjugate to each well. Discard remaining reconstituted Enzyme Conjugate after use.
5. Incubate 60 ± 5 minutes at room temperature (18-28°C).
6. 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.

Substrate Incubation

1. Manually invert/empty strips. Add at least 250 μ L of 1X Wash Buffer 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. While the strips are inverted, carefully wipe the bottom of strips with a lint-free paper towel to ensure that the bottom of the strips are clean.
2. Add 100 μ L of Working Substrate Solution to each well.
3. Incubate for 60 ± 5 minutes at room temperature (18-28°C).

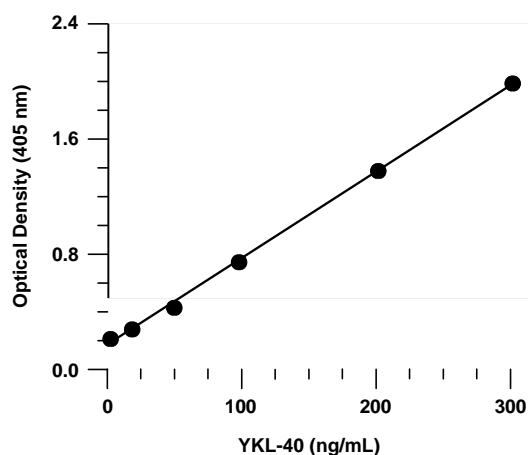
YKL-40 ASSAY PROCEDURE (CONT.)

Stop/Read

1. Add 100 μ L of Stop Solution to each well. Add Stop Solution in the same pattern and time intervals as the Working Substrate Solution addition.
2. Read the Optical Density (OD) 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.
3. Quantitation software with a linear calibration curve fitting equation **must** be used to analyze the YKL-40 assay results.
4. Determine concentration of samples and Controls from the standard curve.
5. Control values should be within the range specified in the Certificate of Analysis supplied with the kit.

Representative Standard Curve

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



INTERPRETATION OF RESULTS

YKL-40 assay results are expressed in ng/mL and **do not** need to be corrected for dilution (unless sample was diluted prior to testing).

YKL-40 EXPECTED VALUES

Preliminary YKL-40 reference ranges have been established for healthy adult males (n=103) and healthy adult females (n=226) ≤ 60 years of age. For the purposes of establishing reference ranges, healthy subjects were defined as:

- Basically healthy, no articular, bone, liver, endocrine or other chronic disorders
- Not currently taking any medication known to modify arthritic disease or influence joint metabolism (e.g. NSAIDs, SAARDs, DMARDs)

Values may be influenced by diseases known to affect tissue remodeling, such as hepatic fibrosis, metastatic carcinoma, osteoarthritis, or rheumatoid arthritis.

Each laboratory should establish its own reference range. The results are expressed as nonparametric reference intervals (90% CI).

Females	25 - 93 ng/mL
Males	24 - 125 ng/mL

PERFORMANCE CHARACTERISTICS

Sensitivity

The minimum detection limit of the YKL-40 assay is 20 ng/mL.

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.

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

Recovery - Linearity

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	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. Samples shown below represent a range of values.

Chondrex (ng/mL)	Within-run ¹ C.V. (%)	Between-run ² C.V. (%)
51.8	6.6	6.8
177.8	5.6	7.0
262.9	5.8	6.0

¹ n = 21 or 22 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

PERFORMANCE CHARACTERISTICS (CONT.)

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

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