ELISA PRODUCT INFORMATION & MANUAL

Rat Lumican ELISA Kit (Colorimetric)

NBP3-20173

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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Protocol

Rat Lumican ELISA Kit (Colorimetric)

Important Note!
The Protocol listed on the website should NOT be used as a final document.
Please refer to the Protocol that is supplied with the kit and specific lot number.

Lumican, also known as LUM, is a protein which in humans is encoded by the LUM gene. This gene is a member of the small interstitial proteoglycan gene(SIPG) family, and it is a keratan sulfate proteoglycan which presents large quantities in the corneal stroma and in interstitial collagenous matrices of the heart, aorta, skeletal muscle, skin, and intervertebral discs. Lumican is mapped to 12q21.33, it interacts with collagen and limits growth of fibrils in diameter. In the cornea, Lumican not only interacts with collagen molecules to limit fibril growth, but also plays a critical role in the regular spacing of fibrils and acquisition of corneal transparency by virtue of its keratan sulfate-containing glycosaminoglycan side chains LDC.

Intended Use:
Rat Lumican ELISA Kit (Colorimetric) is for the quantitative detection of rat Lumican in cell culture supernates, serum and plasma (Heparin).

Test Principle:
Rat Lumican ELISA Kit (Colorimetric) is a solid-phase immunoassay specially designed to measure rat Lumican with a 96-well strip plate that is pre-coated with antibody specific for Lumican. The detection antibody is a biotinylated antibody specific for Lumican. The capture antibody is monoclonal antibody from mouse and the detection antibody is polyclonal antibody from goat. The kit contains recombinant rat Lumican with immunogen: Expression system for standard: NS0 cells; Immunogen sequence: Q19-N338.

To measure rat Lumican, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to rat Lumican in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of rat Lumican in the sample.

Kit Components:
Microtiter Strips, 1x96 wells
*Lumican Standard, 2x10ng
Anti-rat Lumican (Biotin), 1x100ul (1:100 dilution)
Avidin-Biotin-Peroxidase Complex (ABC), 1x100ul (1:100 dilution)
Sample Diluent Buffer, 1x30ml
Antibody Diluent Buffer, 1x12ml
ABC Diluent Buffer, 1x12ml
TMB Color Developing Agent, 1x10ml
TMB Stop Solution, 2N H_2SO_4, 1x10ml
Wash Buffer, 25X, 1x20ml

Storage and Stability:
Store *Lumican Standard powder at 4°C. Once reconstituted store at 4°C for up to 12 hours or at -20°C for up to 48 hours. Store other components at 4°C. Stable for 6 months after receipt. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

Material Required But Not Provided:
1. Microplate reader capable of reading absorbance at 450nm
2. Automated plate washer (optional)
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection
4. Deionized or distilled water
5.Test tubes for dilutions
6. 500ml graduated cylinders

Application Notes:
1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color Developing agent is colorless and transparent before using, please contact us if it is not the case.

3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Duplicate well assay is recommended for both standard and sample testing.
5. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
6. Don't reuse tips and tubes to avoid cross contamination.
7. To avoid to use the reagents from different batches together.
8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 minutes before using.

Sample Preparation and Storage:

1. *Cell Culture Supernates:* Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

2. *Serum:* Allow the serum to clot in a serum separator tube (about 4 hours) at RT. Centrifuge at approximately 1000xg for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

3. *Plasma:* Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at approximately 1000xg. Assay immediately or store samples at -20°C.

**Note:* it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.

Lumican Standard
a) Use one 10ng of standard for each experiment. Reconstitute the Standard with 1ml of Sample Diluent Buffer*:* Sample Diluent Buffer and let stand for 10 minutes at RT; shake gently, taking care not to foam. The concentration of the stock solution is 10,000pg/ml.
b) Prepare 7 tubes containing 300ul Standard Diluent and produce a double dilution series according to the table shown below. Mix each tube thoroughly before the next transfer. Set up 7 points for the standard curve: 10,000pg/ml, 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312pg/ml, 156pg/ml and the last tube with Standard Diluent as the blank at 0pg/ml.

Tube #	Vol. Standard	Vol. Std. Diluent	Final Concentration
Stock	10,000pg (lyo.)	1000ul	10,000pg/ml
1	300ul from Stock	300ul	5000pg/ml
2	300ul from Tube 1	300ul	2500pg/ml
3	300ul from Tube 2	300ul	1250pg/ml
4	300ul from Tube 3	300ul	625pg/ml
5	300ul from Tube 4	300ul	312pg/ml
6	300ul from Tube 5	300ul	156pg/ml
7	0ul	300ul	0pg/ml

Note: The standard solutions are best used within 2 hours.

2. Preparation of Anti-rat Lumican (Biotin)*:* Anti-rat Lumican (Biotin) Working Solution: The solution should be prepared no more than 2 hours prior to the experiment.
a. The total volume should be: 100ul/well x (the number of wells). (Allowing 100-200ul more than total volume)
b. Biotinylated anti-rat Lumican antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1ul Biotinylated anti-rat Lumican antibody to 99ul antibody diluent buffer.)

3. Preparation of Avidin-Biotin-Peroxidase Complex (ABC)*:* Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 2 hours prior to the experiment.
a. The total volume should be: 100ul/well x (the number of wells). (Allowing 100-200ul more than total volume)
b. Avidin-Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC Diluent Buffer*:* ABC Diluent Buffer and mixed thoroughly. (i.e. Add 1ul ABC to 99ul ABC Diluent Buffer.)

4. Preparation of Wash Buffer*:* Wash Buffer, 25X working wash buffer: Prepare 500ml of Working Wash Buffer by diluting 20ml Wash Buffer, 25X with 480ml of deionized or distilled water. If crystals have formed in the concentrate, warm to RT and mix it gently until crystals have completely dissolved.

Assay Procedure:
The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard Lumican detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of Lumican amount in

samples.

1. Aliquot 100ul per well of the 10,.000pg/ml, 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312pg/ml, 156pg/ml human LDLR standard solutions into the precoated 96-well plate. Add 100ul of the sample diluent buffer into the control well (Zero well). Add 100ul of each properly diluted sample of human cell culture supernates, serum or plasma (heparin) or urine to each empty well. It is recommended that each rat Lumican standard solution and each sample be measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 minutes (RT for 2 hours).
3. Remove the cover, discard plate content and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 100ul of biotinylated anti-human LDLR antibody working solution into each well and incubate the plate at 37°C for 60 minutes (RT for 90 minutes).
5. Wash plate 3 times with Working Wash Buffer and each time let washing buffer stay in the wells for 1 minute. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (*Plate Washing Method:* Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 300ul Working Wash Buffer for 1 minute. Repeat this process two additional times for a total of THREE washes. *Note:* For automated washing, aspirate all wells and wash THREE times with Working Wash Buffer, overfilling wells with Working Wash Buffer. Blot the plate onto paper towels or other absorbent material.)
6. Add 100ul of prepared ABC working solution into each well and incubate the plate at 37°C for 30 minutes (RT for 40 minutes).
7. Wash plate 5 times with Working Wash Buffer and each time let washing buffer stay in the wells for 1-2 minutes. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90ul of TMB Color Developing Agent*:* TMB Color Developing Agent into each well and incubate plate at 37°C in dark for 15-25 minutes (RT for 30 minutes). (*Note:* For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated rat Lumican standard solutions; the other wells show no obvious color).
9. Add 100ul of TMB Stop Solution, 2N H2SO4*:* TMB Stop Solution, 2N H2SO4 into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 minutes after adding the stop solution.

Calculations:
Average the duplicate readings for each standard and sample. Subtract the average zero standard O.D. reading.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The rat Lumican concentration of the samples can be interpolated from the standard curve.
Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical Data:
Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. vlaue is not less than 1.0.

Concentration (pg/ml)	O.D.
0.0	0.129
156	0.224
312	0.293
625	0.462
1250	0.697
2500	1.123
5000	1.554
10,000	2.144

Range: 156-10,000pg/ml
Sensitivity: <10pg/ml
Specificity: Recognizes natural and recombinant rat Lumican. There is no detectable cross-reactivity with other relevant proteins.

Precision:
Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision.

Intra-Assay Precision				Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (pg/ml)	202	1179	5476	186	1201	5528
Standard Deviation	13.33	54.23	339.51	16.65	58.84	414.6
CV (%)	6.6	4.6	6.2	8.2	4.9	7.5

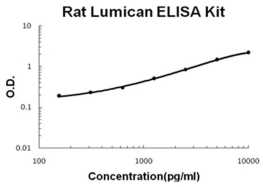
Reproducibility:
To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots (pg/ml)	1	2	3	4	Mean	Std Deviation	CV(%)
Sample1	202	232	199	227	215	14.64	6.8
Sample 2	1179	1091	1283	1296	1212	83.4	6.8
Sample 3	5476	4974	5363	5047	5215	209.95	4.0

*Number of samples for each test n=16.

Assay Summary:
1. Add 100ul of samples and standards and incubate the plate at 37°C for 90 minutes or at RT for 2 hours. Do not wash.
2. Add 100ul biotinylated antibody and incubate the plate at 37°C for 60 minutes or at RT for 90 minutes. Wash plate 3 times with Working Wash Buffer.
3. Add 100ul of ABC working solution and incubate the plate at 37°C for 30 minutes or at RT for 40 minutes. Wash plate 5 times with Working Wash Buffer.
4. Add 90ul of TMB color developing agent and incubate the plate at 37°C in dark for 15-25 minutes or at RT for 30 minutes.
5. Add 100ul TMB Stop Solution and read.

Images



Typical standard curve for Rat Lumican ELISA Kit (Colorimetric) (shown for reference only; user must generate a new standard curve with each assay).