COLL2-1NO_2 ELISA Kit

Catalog Number KA2098

96 assays

Version: 03

Intended for research use only

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Introduction

Background

This immunoassay allows measuring in urine a degradation fragment containing a 9 amino acid sequence (HRGYPGLDG) which is nitrated on its tyrosine residue [HRGY(NO_2)PGLDG]. This degradation fragment, named Coll2-1-NO_2, is derived from type II collagen and is released into the synovial fluid following the action of collagenases and gelatinase B. Subsequent tyrosine nitration results from the action of peroxynitrite (ONOO-), a strong oxidant generated during the inflammatory process.

Principle of the Assay

This assay is a competitive immunoassay utilizing a synthetic peptide pre-coated onto the ELISA plate (PLT) for the quantitation of the corresponding peptide antigen in urine samples. A binding competition between the immobilized peptide and the peptide contained in the standards (STD) or samples takes place upon addition of the antibody Ab-Coll2-1-NO_2 (AB1). After removal of the unbound peptide, a peroxidase-conjugated goat anti-rabbit antibody (AB2) is added into each well to detect and quantify the level of competitive binding. After washing of the unbound detection antibody, the antibody-antigen complex is detected by a chromogenic reaction with 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid (STOP) to give a colorimetric endpoint that is subsequently determined spectrophotometrically.

General Information

Materials Supplied

List of component

Component	Amount
PLT: Coll2-1NO_2 pre-coated Microplate.	96 wells
STD: Coll2-1NO_2 standard, 4 nM calibrator.	500 µL
CTL1: Positive control, 2 nM (Ready-to-Use).	250 µL
CTL2: Positive control, 0.4 nM (Ready-to-Use).	250 µL
AB1: Coll2-1NO_2 polyclonal antibody stock solution.	50 µL
AB2: Peroxidase-conjugated goat anti-rabbit antibody stock solution.	150 µL
DIL: Diluent for STD, samples, AB1 and AB2.	35 mL
WASH: Wash buffer to be diluted 10x in H_2O	40 mL x 2
TMB: 3,3',5,5'-tetramethylbenzidine (Ready-to-Use).	15 mL
STOP: H_2SO_4 0.5 M (Ready-to-Use)	15 mL

Storage Instruction

All reagents should be stored at 2-8°C for stability until the expiry date printed.

Materials Required but Not Supplied

- ✓ Pipettors to deliver 10 - 1000 µL
- ✓ Repeating or multichannel pipettor to deliver 10 - 1000 µL
- ✓ Pipettes to deliver 10 – 25 mL
- ✓ Sample tube vortex
- ✓ Microtiter plate shaker (600 rpm)
- ✓ Microtiter plate washer (preferred – manual wash steps are allowed)
- ✓ Microtiter plate reader with dual wavelength reading at 450 nm and 650 nm
- ✓ Deionized water and glassware

Note: Ensure that the equipment is calibrated and in proper working order.

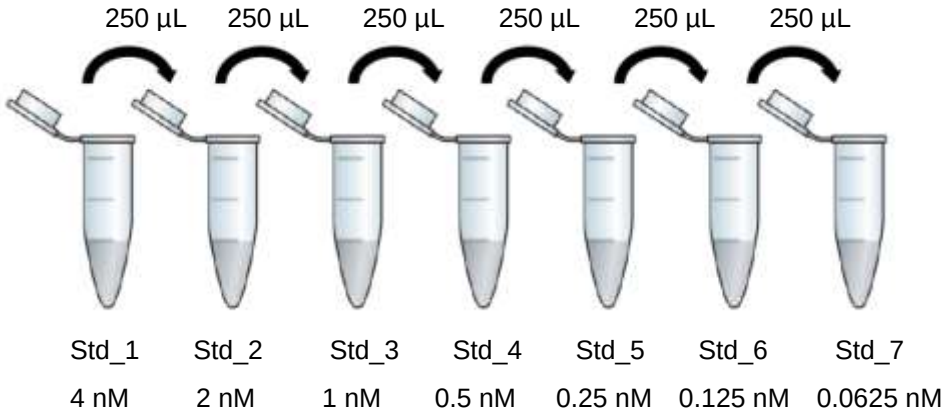
Assay Protocol

Reagent Preparation

✓ Standard

Please prepare the standard following the table below:

Table 1: Coll2-1-NO_2 Working Standards Dilution				
Standard	**Concentration**	**Volume Stock**	**Volume Buffer**	**Dilution**
Std_1	4 nM	500 µL	STD (Ready-to-Use)	
Std_2	2 nM	250 µL	250 µL	1:2
Std_3	1 nM	250 µL	250 µL	1:2
Std_4	0.5 nM	250 µL	250 µL	1:2
Std_5	0.25 nM	250 µL	250 µL	1:2
Std_6	0.125 nM	250 µL	250 µL	1:2
Std_7	0.0625 nM	250 µL	250 µL	1:2



Assay Procedure

1. Place wash buffer (WASH) 10 minutes at 37°C to ensure total solubilization then mix the bottle gently. Bring all other reagents to room temperature.
2. Prepare at least 600 mL of washing buffer (1x) per plate (e.g. for 600 mL of washing buffer dilute 60 mL of stock WASH to 600 mL with water).
3. Wash the PLT 4 times by adding 350 µL of washing buffer (1x) per well. After the last washing step, tap firmly the plate several times over clean absorbent paper until any residual liquid is removed. Do not leave the plate to dry for more than 5 minutes.
4. Briefly vortex the STD, CTL1 and CTL2 then collect any liquid by centrifugation.
5. Prepare 7 standards by dilution in Diluent (DIL) from the stock standard at 4 nM as described above. Prepare 6 tubes containing 250 µL DIL and produce the following dilution series: 4 nM (undiluted), 2 nM, 1 nM, 0.5 nM, 0.25 nM, 0.125 nM and 0.0625 nM. Ensure that each tube is thoroughly mixed before the

next transfer.

6. Distribute at least 50 µL of standards, controls, and samples at least in duplicate into the plate PLT. Prepare B0 (zero calibrator) by adding 50 µL of DIL in duplicate. Typical plate disposition of standards (Std, B0), Unknown samples (Unk) and Controls (CTL) please the plate layout.
7. Prepare the AB1 working solution with DIL (e.g. for one plate, add 30 µL of AB1 stock solution to 12 mL of DIL).
8. Add 100 µL of AB1 working solution into each well.
9. Cover the plate with sealing tape and incubate on plate shaker (600 rpm) for 1 hour at room temperature.
10. Wash the plate 4 times with washing buffer (1x) as described in step 3.
11. Prepare the AB2 working solution with DIL (e.g. for one plate, add 120 µL of AB2 stock solution to 12 mL of DIL).
12. Add 100 µL of AB2 working solution into each well.
13. Cover the plate with sealing tape and incubate on plate shaker (600 rpm) for 1 hour at room temperature.
14. Wash the plate 4 times with washing buffer (1x) as described in step 3.
15. Add 100 µL of the TMB into each well.
16. Cover the plate with sealing tape and incubate on plate shaker (600 rpm) for 15 minutes at room temperature.
17. Add 100 µL of STOP into each well.
18. Measure the absorbance at 450 nm with 650 nm as reference within 20 min. Avoid light exposure until the reading.

Note:

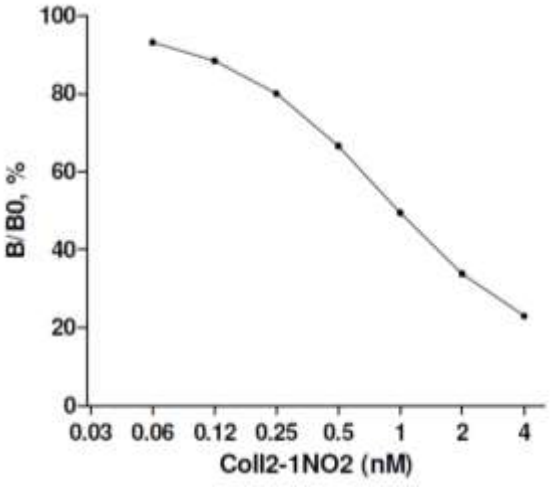
1. *Before modifying the recommended protocol for any reason, users are encouraged to contact our technical services to achieve the desired goals.*
2. *All standards, controls & samples should be tested at least in duplicate*
3. Plate reader should be turned on 30 minutes before reading to allow pre-heating.
4. Plates should be read within 20 minutes since color will fade away over time.

Data Analysis

Calculation of Results

1. Calculate %B/B0 where B is the coated antigen bound in the presence of free antigen and B0 is the coated antigen bound in the zero calibrator. Plot %B/B0 on the y-axis versus log concentration on the axis. Analyze the standard curve using 4-Parameter Logistic regression with appropriate curve-fitting software.
2. Determine the Coll2-1NO_2 concentration of each urine sample by interpolation onto the regression model. Note that a high B/B0 ratio corresponds to a low concentration of Coll2-1NO_2 in the analyzed sample.

✓ **Typical Standard Curve**



Standard #	Concentration
Std_1	4 nM
Std_2	2 nM
Std_3	1 nM
Std_4	0.5 nM
Std_5	0.25 nM
Std_6	0.125 nM
Std_7	0.0625 nM

Figure 1: Typical Standard Curve for COLL2-1NO_2 ELISA Kit.

✓ QUALITY CONTROL

1. Precision on absorbance duplicates should yield %coefficients of variation ≤ 15%.
2. The %B0 of 4 nM Std_1 (CAL) should be less than or equal to 40%
3. CTL1 and CTL2 Controls should be 1.3-2.7 nM and 0.25-0.55 nM, respectively.
4. Acceptable sample values should fall within the working range of the standard curve. Any sample higher than 4 nM should be diluted further and re-assessed. Any sample lower than 0.0625 nM should be re-assessed or discarded

Performance Characteristics

✓ Specificity

No cross-reactivity is observed between Coll2-1NO_2 and human nitrated type I and II collagens, human native type I and II collagens, human heat denatured nitrated type I and II collagens, nitrated BSA, native BSA and NO_2-tyr.

- ✓ Matrix interference

  Interference of the following urine constituents is found not to affect the assay: lipids (up to 5 g/L) and bilirubin (up to 250 µM).

- ✓ Detection limit

  The limit of detection of this assay is 0.06 nM.

- ✓ Working range

  The range of quantification of this assay is 0.06 nM-4 nM.

- ✓ Precision

  The intra-/inter-assay % coefficients of variation of this assay are ≤ 20%.

- ✓ Dilutional linearity & Spiking recovery

  Dilutional linearity is of 100 ± 20% in a sample dilution range from 2 to 4-fold. Spike recovery is of 100 ± 20% in urine samples.

Resources

References

1. New serum biochemical markers (Coll 2-1 and Coll 2-1 NO_2) for studying oxidative related type II collagen network degradation in patients with osteoarthritis and rheumatoid arthritis. M. Deberg, A. Labasse, S. Christgau, P. Cloos, D.B. Henriksen, J.P. Chapelle, B. Zegels, Y. Reginster and Y. Henrotin. Osteoarthritis and Cartilage, 13: 258-265 (2005).
2. Type II collagen peptides for measuring cartilage degradation. Y. Henrotin, M. Deberg, J.E. Dubuc, E. Quettier, S. Christgau, J.Y.Reginster. Biorheology 41: 543-547 (2004).

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std_1	Std_1	Unk_1	Unk_1	Unk_9	Unk_9	Unk_17	Unk_17	Unk_25	Unk_25	Unk_33	Unk_33
B	Std_2	Std_2	Unk_2	Unk_2	Unk_10	Unk_10	Unk_18	Unk_18	Unk_26	Unk_26	Unk_34	Unk_34
C	Std_3	Std_3	Unk_3	Unk_3	Unk_11	Unk_11	Unk_19	Unk_19	Unk_27	Unk_27	Unk_35	Unk_35
D	Std_4	Std_4	Unk_4	Unk_4	Unk_12	Unk_12	Unk_20	Unk_20	Unk_28	Unk_28	Unk_36	Unk_36
E	Std_5	Std_5	Unk_5	Unk_5	Unk_13	Unk_13	Unk_21	Unk_21	Unk_29	Unk_29	Unk_37	Unk_37
F	Std_6	Std_6	Unk_6	Unk_6	Unk_14	Unk_14	Unk_22	Unk_22	Unk_30	Unk_30	Unk_38	Unk_38
G	Std_7	Std_7	Unk_7	Unk_7	Unk_15	Unk_15	Unk_23	Unk_23	Unk_31	Unk_31	CTL1	CTL1
H	B0	B0	Unk_8	Unk_8	Unk_16	Unk_16	Unk_24	Unk_24	Unk_32	Unk_32	CTL2	CTL2