Datasheet for ABIN6963330

Collagen IV ELISA Kit

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Overview

Quantity:	96 tests
Target:	Collagen IV (COL4)
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	0.78 ng/mL - 50 ng/mL
Minimum Detection Limit:	0.78 ng/mL
Application:	ELISA

Product Details

Purpose:	The kit is a sandwich enzyme immunoassay technique for the in vitro quantitative measurement in various sample types.
Sample Type:	Cell Culture Supernatant, Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This kit recognizes Rat COL4 in samples. No significant cross-reactivity or interference between Rat COL4 and analogues was observed.
Sensitivity:	0.47 ng/mL
Components:	• Pre-coated, ready to use 96-well strip plate, flat buttom • Plate sealer for 96 wells • Reference Standard • Reference Standard & Sample Diluent

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Product Details

- Biotinylated Detection Antibody (100 x concentrate)
- HRP Conjugate (100 x concentrate)
- Biotinylated Detection Antibody Diluent
- HRP Conjugate Diluent
- Substrate Reagent
- Stop Solution
- Wash Buffer (25 x concentrate)
- Instruction manual

Target Details

Target:	Collagen IV (COL4)
Alternative Name:	Collagen Type IV (COL4 Products)
Background:	Type-IV Collagen

Application Details

Sample Volume:	100 µL
Assay Time:	3.5 h
Plate:	Pre-coated
Protocol:	1. Add 100 µL standard or sample to each well. Incubate for 90 min at 37 °C. 2. Remove the liquid. Add 100 µL Biotinylated Detection Antibody. Incubate for 1 hour at 37 °C. 3. Aspirate and wash 3 times. 4. Add 100 µL HRP Conjugate. Incubate for 30 min at 37 °C. 5. Aspirate and wash 5 times. 6. Add 90 µL Substrate Reagent. Incubate for 15 min at 37 °C. 7. Add 50 µL Stop Solution. Read at 450 nm immediately. 8. Calculation of results.
Reagent Preparation:	1. Bring all reagents to room temperature (18-25 °C) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition. 2. Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40 °C water bath and mix it gently until the crystals have completely dissolved. 3. Standard working solution: Centrifuge the standard at 10,000xg for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 50 ng/mL(or add 1.0 mL of Reference Standard &Sample Diluent, let it

stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles generated during vortex could be removed by centrifuging at a relatively low speed). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 50, 25, 12.5, 6.25, 3.13, 1.57, 0.78, 0 ng/mL. Dilution method: Take 7 EP tubes, add 500μL of Reference Standard & Sample Diluent to each tube. Pipette 500μL of the 50 ng/mL working solution to the first tube and mix up to produce a 25 ng/mL working solution. Pipette 500μL of the solution from the former tube into the latter one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.

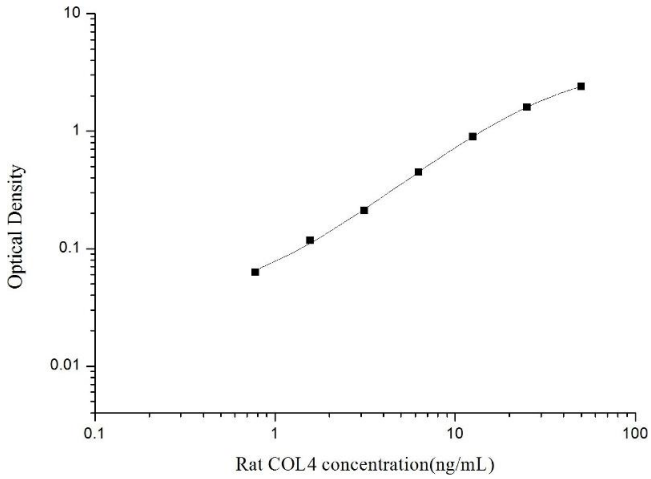
4. Biotinylated Detection Antibody working solution: Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Antibody at 800xg for 1 min, then dilute the 100x Concentrated Biotinylated Detection Antibody to 1x working solution with Biotinylated Detection Antibody Diluent(Concentrated Biotinylated Detection Antibody: Biotinylated Detection Antibody Diluent= 1: 99).
5. HRP Conjugate working solution: Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800xg for 1 min, then dilute -6- the 100x Concentrated HRP Conjugate to 1x working solution with HRP Conjugate Diluent(Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99). 50 25 12.5 6.25 3.13 1.57 0.78 0

Sample Preparation:	• It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates. • If the sample type is not specified in the instructions, a preliminary test is necessary to determinecompatibility with the kit. • If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibilityof causing a deviation due to the introduced chemical substance.The recommended dilution factor is for reference only. • Please estimate the concentration of the samples before performing the test. If the values are not in therange of the standard curve, the optimal sample dilution for the particular experiment has to be determined.Samples should then be diluted with PBS (pH =7.0-7.2).
Assay Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level Rat COL4 were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level Rat COL4 were tested on 3 different plates, 20 replicates in each plate. Both intra-CV and inter-CV are < 10 %.
Restrictions:	For Research Use only

Handling

Storage:	4 °C,-20 °C
Storage Comment:	1. For unopened kit: All reagents should be stored according to the labels on the vials, so they are stable up to 6 months after receipt of the kit. The Reference Standard, Biotinylated Detection Antibody, HRP Conjugate and the 96-well stripe plate should be stored at -20 °C upon receipt while the other reagents should be stored at 4 °C. 2. For used kit: When the kit is used, the remaining reagents need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and zip-seal the foil pouch.
Expiry Date:	6 months

Images



ELISA

Image 1. Typical standard curve

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