Datasheet for ABIN6963322

CTXII ELISA Kit

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Overview

Quantity:	96 tests
Target:	CTXII (CTX-II)
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	0.16 ng/mL - 10 ng/mL
Minimum Detection Limit:	0.16 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 CTX-II in samples. No Significant cross-reactivity or interference between Rat CTX-II and analogues was observed.
Sensitivity:	0.1 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

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:	CTXII (CTX-II)
Alternative Name:	Cross Linked C-telopeptide of Type II Collagen (CTX-II Products)

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. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement. 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 10 ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 10, 5, 2.5, 1.25, 0.63, 0.32, 0.16, 0 ng/mL. Dilution method: Take 7 EP tubes, add 500 µLof Reference Standard & Sample Diluent to each tube. Pipette 500 µ Lof the 10 ng/mL working solution to the first tube and mix up to produce a 5 ng/mL working

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Application Details

solution. Pipette 500 µLof the solution from the former tube into the latter one according to these steps. 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 stock tube before use, dilute the 100x Concentrated Biotinylated Detection Antibody to 1xworking solution with Biotinylated Detection Antibody Diluent.
5. Concentrated HRP Conjugate working solution: Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100x Concentrated HRP Conjugate to 1x working solution with Concentrated HRP Conjugate Diluent.

Sample Preparation:	• It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturation may occur in these samples, leading to false results. Samples should therefore be stored for a short period at 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thaw cycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates. • If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit. • If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of 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 the range of the standard curve, the optimal sample dilution for the particular experiment has to be determined.
Assay Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level Rat CTX-II were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level Rat CTX-II 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 12 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

Handling

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:	12 months

Publications

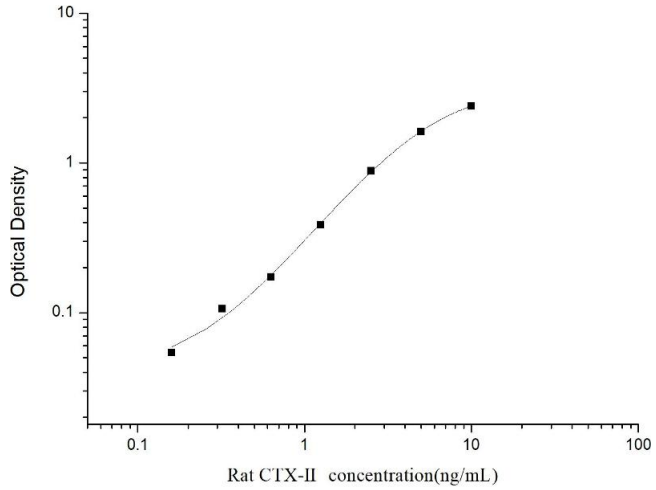
Product cited in:

Zhang, Fu, Chen, Chen, Yang: "Protocatechuic acid attenuates anterior cruciate ligament transection-induced osteoarthritis by suppressing osteoclastogenesis." in: **Experimental and therapeutic medicine**, Vol. 19, Issue 1, pp. 232-240, (2020) (PubMed).

Kothari, Sinha, Sardar, Tripathi, Girme, Adhikary, Singh, Maurya, Mishra, Hingorani, Trivedi: " Inhibition of cartilage degeneration and subchondral bone deterioration by Spinacia oleracea in human mimic of ACLT-induced osteoarthritis." in: **Food & function**, Vol. 11, Issue 9, pp. 8273-8285, (2020) (PubMed).

Choudhary, Kothari, Tripathi, Singh, Adhikary, Ahmad, Kumar, Dev, Mishra, Shukla, Maurya, Mishra, Trivedi: "Spinacia oleracea extract attenuates disease progression and sub-chondral bone changes in monosodium iodoacetate-induced osteoarthritis in rats." in: **BMC complementary and alternative medicine**, Vol. 18, Issue 1, pp. 69, (2018) (PubMed).

Images



ELISA

Image 1. Typical standard curve