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Datasheet for ABIN5591838 CKB ELISA Kit



Overview

Quantity:	96 tests
Target:	СКВ
Reactivity:	Human
Method Type:	Competition ELISA
Detection Range:	0.156-10 ng/mL
Minimum Detection Limit:	0.156 ng/mL
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the in vitro quantitative determination of human Creatine kinase B-type concentrations in serum, plasma, tissue homogenates, cell culture supernates, and other biological fluids.
Sample Type:	Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural Human Creatine kinase B-type. No significant cross-reactivity or interference was observed. Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between human Creatine kinase B-type and all the analogues, therefore, cross reaction may still exist.
Sensitivity:	< 0.078 ng/mL

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Product Details

Components:	Assay plate x1
	Standard x2
	Sample Diluent 1 x 20ml
	Assay Diluent A 1 x 10ml
	Assay Diluent B 1 x 10ml
	Detection Reagent A 1 x 60µl
	Detection Reagent B 1 x 120µl
	Wash Buffer(25 x concentrate) 1 x 30ml
	Substrate 1 x 10ml
	Stop Solution 1 x 10ml
	Plate sealer for 96 wells 5 instructions 1
Material not included:	Microplate reader.
	Pipettes and pipette tips.
	EP tube Deionized or distilled water.

Target Details

Target:	СКВ
Alternative Name:	CKB (CKB Products)
Background:	Synonyms: Creatine kinase B-type, B-CK,Creatine kinase B chain
UniProt:	P12277

Application Details

Comment:	 We are only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance. The kit should not be used beyond the expiration date on the kit label. Do not mix or substitute reagents with those from other lots or sources. If samples generate values higher than the highest standard, further dilute the samples with the Sample Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
Sample Volume:	50 µL
Assay Time:	3 - 5 h
Plate:	Pre-coated

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Application Details

Protocol:	The ELISA is based on the competitive binding enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Creatine kinase B-type, During the reaction, Creatine kinase B-type in the sample or standard competes with a fixed amount of biotin-labeled Creatine kinase B-type for sites on a pre-coated Monoclonal antibody specific to Creatine kinase B-type. Excess conjugate and unbound sample or standard are washed from the plate. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of Creatine kinase B-type in the samples is then determined by comparing the
	O.D. of the samples to the standard curve.
Reagent Preparation:	 Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 10.0 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). The undiluted standard serves as the high standard (10.0 ng/mL). Detection Reagent A and B - Dilute to the working concentration using Assay Diluent A and B (1:100), respectively.
Sample Collection:	 Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20 °C or -80 °C. Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2 - 8 °C within 30 minutes of collection. Store samples at -20 °C or -80 °C. 80 °C. Avoid repeated freeze-thaw cycles. Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissue was rinsed with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS and stored overnight at ≤ -20 °C. After two freeze-thaw cycles were performed

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to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. Remove the supernate and assay immediately or aliquot and store at \leq -20 °C. Cell culture

supernates and other biological fluids - Remove particulates by centrifugation and assay

immediately or aliquot and store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles.

Note:

- 1. Samples to be used within 5 days may be stored at 2-8 °C , otherwise samples must stored at -20 °C (1 month) or -80 °C (2 months) to avoid loss of bioactivity and contamination.
- 2. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
- 3. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit
- 4. Sample hemolysis will influence the result, so hemolytic specimen can not be detected.
- 5. When performing the assay slowly bring samples to room temperature.
- 6. Do not use heat-treated specimens.

Assay Procedure: Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37 °C directly.). All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4 °C until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

- 1. Add 50 μL of Standard, Blank, or Sample per well.
- 2. Immediately add 50 μ L of Detection A working solution to each well. Cover with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37 °C.
- 3. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 μL) using a squirt bottle, multichannel pipette, manifold dispenser or autowasher and let it sit for 1~2 minutes. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
- 4. Add 100 μ L of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for 45 minutes at 37 °C.
- 5. Repeat the aspiration/wash process for five times as conducted in step three.
- 6. Add 90 μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate within 15-30 minutes at 37 °C. Protect from light.
- 7. Add 50 μ L of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
- 8. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

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	 Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required stripwells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards Detection Reagent A and B can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10 µL for once pipetting. To ensure accurate results, proper adhesion of plate sealers during incubation steps is
	necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay.
	For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
	 To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
	The wash procedure is critical. insufficient washing will result in poor precision and falsely elevated absorbance readings.
	 Duplication of all standards and specimens, although not required, is recommended. Substrate Solution is easily contaminated. Please protect it from light.
	9. The web version of manual is only for reference, subject to the instruction shipping with the kit.
Calculation of Results:	Average the duplicate readings for each standard, control, and sample.Create a standard curve
	by reducing the data using computer software capable of generating a four parameter logistic
	(4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance
	for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve
	through the points on the graph. The data may be linearized by plotting the log of the Creatine
	kinase B-type concentrations versus the log of the O.D. and the best fit line can be determined
	by regression analysis. It is recommended to use some related software to do this calculation,
	such as curve expert 1.3. This procedure will produce an adequate but less precise fit of the
	data. If samples have been diluted, the concentration read from the standard curve must be
	multiplied by the dilution factor.

Important note:

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 Do not remove microtiter plate from the storage bag until needed. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-
3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement. 7. Use fresh disposable pipette tips for each transfer to avoid contamination.
8. Do not substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
 9. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended. 10. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too. 11. Kits from different manufacturers for the same item might produce different results, since we haven't compared our products with other manufacturers. 12. The instruction manual also suit for the kit of 48T, but all reagents of 48T kit is reduced by half. 13. Valid period: six months.
The Oten Calution supported for use with this bit is an exidentian Ween ave band for and
The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
4 °C
The Assay Plate, Standard, Detection Reagent A and Detection Reagent B should be stored at -
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Note: Because of the inherent stability of lyophilized materialthese materials may shipped at ambient temperature.

Expiry Date:

6 months

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