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Datasheet for ABIN1125223 CPS1 ELISA Kit

Validation



Overview

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| Quantity: | 96 tests |
|----------------------|---------------|
| Target: | CPS1 |
| Binding Specificity: | Mitochondrial |
| Reactivity: | Human |
| Application: | ELISA |

Product Details

| Detection Method: | Colorimetric |
|-------------------|--|
| Characteristics: | Homo sapiens,Human,Carbamoyl-phosphate synthase [ammonia], mitochondrial,Carbamoyl- phosphate synthetase I,CPSase I,CPS1,6.3.4.16 |
| Components: | Reagent (Quantity): Assay plate (1), Standard (2), Sample Diluent (1x20ml), Assay Diluent A (1x10ml), Assay Diluent B (1x10ml), Detection Reagent A (1x120µl), Detection Reagent B (1x120µl), Wash Buffer(25 x concentrate) (1x30ml), Substrate (1x10ml), Stop Solution (1x10ml) |

Target Details

| Target: | CPS1 |
|-------------------|---|
| Alternative Name: | CPS1 (CPS1 Products) |
| Background: | Synonyms: Carbamoyl-phosphate synthase [ammonia], mitochondrial,Carbamoyl-phosphate synthetase I,CPS1,CPSase I,Homo sapiens,Human |
| Pathways: | Response to Growth Hormone Stimulus, Cellular Glucan Metabolic Process |

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Application Details

| Comment: | Gene Name: CPS1 |
|------------------|--|
| Sample Volume: | 100 µL |
| Plate: | Pre-coated |
| Restrictions: | For Research Use only |
| Handling | |
| Storage: | 4 °C/-20 °C |
| Storage Comment: | The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon being received. The other reagents can be stored at 4 °C. |



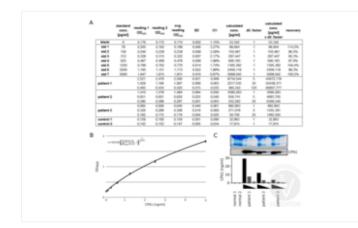
| VALIDATION CUSTOMER VALIDATION | Successfully validated (ELISA (ELISA)) | |
|-----------------------------------|---|--|
| | by Omary Lab, Department of Molecular & Integrative Physiology, University of Michigan | |
| | Medical School | |
| | Report Number: 103213 | |
| | Date: Jun 11 2018 | |
| 103219 11/06/18 | | |
| Target: | CPS1 | |
| Lot Number: | 7K295N | |
| Method validated: | ELISA (ELISA) | |
| Positive Control: | Human serum from patients with liver injury | |
| Negative Control: | Human serum from patients without liver injury | |
| Notes: | Passed, the CPS1 ELISA kit ABIN1125223 specifically detects the antigen in human serum | |
| | samples. | |
| Protocol: | Freeze human serum samples in aliquots until use. Dilute sera with PBS. | |
| | Allow all reagents to reach RT and prepare all the reagents, standard solutions and samples | |
| | as instructed by the manufacturer. | |
| | Add 100µl of standard, blank or sample per well. Cover the plate. Incubate for 2h at 37°C. Remove the liquid. | |
| | Add 100µl detection reagent A. Mix gently and cover the plate. Incubate for 1h at 37°C. | |
| | • Aspirate liquid and wash the wells 3x with 400µl wash buffer. Let it sit for 1-2min. Remove | |
| | liquid Completely at each step. | |
| | Add 100µl of detection reagent B. Cover the plate. Incubate for 1h at 37°C. | |
| | • Wash well 5x with 400µl wash buffer. | |
| | Add 90µl substrate solution. Cover the plate. Incubate 15min at 37°C protected from light. Add 50µl stop solution. Gently tap the plate to ensure thorough mixing. | |
| | Read absorbance at 450nm immediately. | |
| Evnerimental Notes | The purpose of this report is to validate the measurement of human CPS1 in human serum | |
| Experimental Notes: | samples, since it has been published that CPS1 is released into serum during liver injury, | |
| | while it is not detected in healthy human serum (Weerasinghe et al. (2014)). | |
| | • To validate this ELISA, human serum samples previously validated to contain CPS1 by | |
| | Western blotting were used. The signal in the positive controls samples (patients' samples | |
| | that contain high amount of CPS1 which was confirmed by Western blotting) was | |
| | significantly higher than the blank whereas the signal from the negative control samples | |
| | (healthy human sera in that CPS1 was not detected by Western blotting) was comparable to | |
| | the baseline. 1:5, 1:25, or 1:125 diluted serum samples were tested as well. | |
| | CPS1 was not detected in the sera of healthy human by both western blotting and ELISA | |

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method (lane 1 and 2). However, it is highly increased in the sera of patients with acute liver failure and the signals were in a dilution-dependent manner (lane 3 to 11).

 The standard curve for the assays reported were fitted with a 4-Parameter Logistic model. The calculated concentrations of standards were higher than expected, but it gave a good quality fits (R²=0.999). The results showed no CPS1 in the sera from healthy human, while it is highly increased in the sera from patients with liver injury, as expected. Although most of the samples were in the detection range, the calculated concentrations were highest in the most diluted samples.

Image for Validation report #103213



Validation image no. 1 for Carbamoyl-Phosphate Synthase 1, Mitochondrial (CPS1) ELISA Kit (ABIN1125223)

A. Standard curve and sample measurements using
ABIN1125223. B. The standard curves for the assays
reported were fitted with a 4-Parameter Logistic model,
giving good quality fits (R²=0.999). C. CPS1 level
comparison determined by western blotting and
ABIN1125223. Normal serum from healthy humans was
used undiluted. Serum from patient 1 was used undiluted,
1:5, and 1:25 diluted (middle panel). Sera from patient 2 and
patient 3 were used at 1:5, 1:25, and 1:125 dilutions. 2µl of
serum was used for Western blotting; presence of proteins
was verified by Commassie staining (upper panel). The
graph illustrates the ELISA results.

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