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User's Manual

Caffeine ELISA Kit

REF **DEIAPYC4558**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This Caffeine ELISA Kit is a competitive-based ELISA that can quantify caffeine in urine, saliva and serum samples.

General Description

II. Caffeine (1,3,7-trimethylxanthine) is a methylxanthine alkaloid which acts as a stimulant for central nervous system that can be used to increase blood pressure and reduce fatigue. Caffeine is naturally present in leaves, seeds, nuts and a number of plants. It is also known to have anti-inflammatory effect by inhibiting adenosine monophosphate phosphodiesterase. Nowadays, many common beverages including coffee, tea, sodas and energy drinks contain different levels of caffeine to relieve drowsiness and improve performance. At normal dose, caffeine generally improves reaction time, wakefulness, concentration and motor coordination. However, caffeine overdose in the body can lead to nervousness, bone loss, headache, anxiety, insomnia and even death.

Reagents And Materials Provided

ELISA Microplate, 8 X 12 Strips

Standard, 2 vials

HRP Conjugate Stock, 8µl

Antibody, 7ml

TMB Substrate, 12ml

Stop Solution, 10ml

Sample Diluent, 20ml

Wash Buffer (10X), 50ml

Serum Solution, 1.7ml

Standard Buffer, 25ml

Conjugate Buffer, 7.5ml

Plate Sealers, 4

Materials Required But Not Supplied

• Microplate reader capable of measuring absorbance at 450 and 650 nm

• Precision pipettes with disposable tips

• Clean eppendorf tubes for preparing standards or sample dilutions

Storage

The entire kit can be stored at -20°C for up to 12 months from the date of shipment. Opened kit is stable for 1 month at -20°C.

Specimen Collection And Preparation

• Serum

1. Add 20 µl of Serum Solution into 180 µl of serum in an Eppendorf tube and vortex well.
2. Incubate the sample at 37°C for 45 min.
3. After the incubation at 37°C, incubate the sample at 85-90°C for 10 min
4. Dilute the sample 40 fold using the Sample Diluent. (For example, mix 5 µl of serum with 195 µl of Sample Diluent.)
5. Use 50 µl per well for the assay. Note: Dilution factor: 40

• Urine and Saliva

1. Centrifuge 0.5 ml of urine or 0.2 ml of saliva at 10,000 g for 5 min and recover the supernatant.
2. Dilute the supernatant 40 fold using the Sample Diluent. (For example, mix 5 µl of urine with 195 µl of Sample Diluent.)
3. Use 50 µl per well for the assay. Note: Dilution factor: 40

Reagent Preparation

Bring all reagents to room temperature or 4°C before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

• Antibody, TMB Substrate, Stop Solution, Sample Diluent, Serum Solution, Standard Buffer and Conjugate Buffer: Ready to be used. After use, store them at 4°C.

• HRP Conjugate Stock: Spin briefly before opening the tube. Pipette 2 µl of HRP Conjugate Stock into Conjugate Buffer bottle to prepare conjugate working solution. Vortex the conjugate solution bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.

• Wash Buffer (10X): Bring bottle to room temperature. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer with 90 ml deionized water. Concentrated and Diluted Wash Buffer can be stable at 4°C for 3 months.

• Standard: Add 1.5 ml of Standard Buffer into a vial of Caffeine Standard to make S5 standard (27 ng/ml). Perform 3-fold serial dilutions from S5 (e.g. 500 µl S5 in 1 ml of Standard Buffer) to prepare S4 to S1 standards sequentially. S0 contains Standard Buffer only. Diluted standards can be stored at -20°C for 2 weeks.

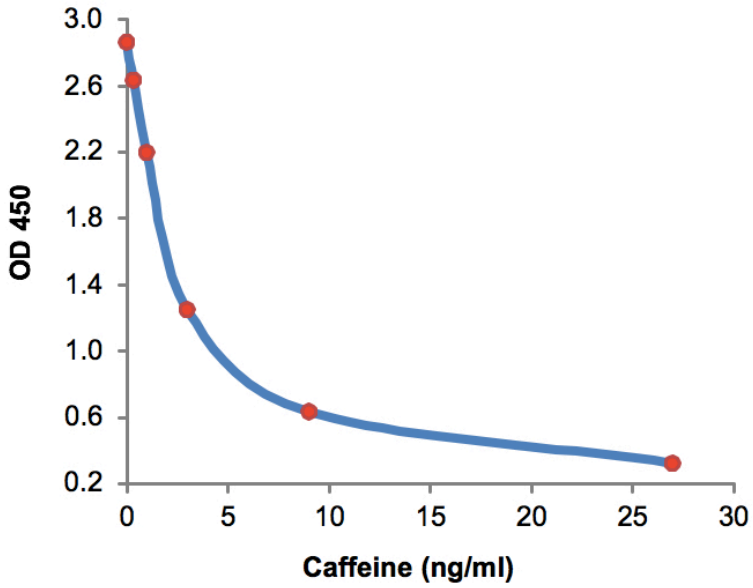
Assay Procedure

Notes: It is recommended that all standards and samples should be run at least in duplicate. Standard curves must be run each time as reference for sample quantification.

1. Prepare all reagents, standards and samples as sections VII and VIII respectively.

2. Add 50 µl of Standards or Samples per well. Add 50 µl of conjugate working solution and 50 µl of Antibody to all wells containing standard or sample.
3. Cover the microtiter plate with plate sealer and mix well. Incubate the plate at room temperature (25°C) for 45 min.
4. Aspirate all reagents and wash each well 4 times: add 250 µl of 1X Wash Buffer and incubate for 30 seconds. Remove 1X Wash buffer completely before the next wash. (This is essential for accurate results.) Repeat this step 3 more times.
5. Add 100 µl of TMB Substrate to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no caffeine (S0). When its reading is approximately between 0.8 and 1.0 (usually between 5-30 min after addition of TMB Substrate), add 50 µl of Stop Solution and gently tap the plate to ensure thorough mixing.
7. Measure OD at 450 nm for the standards and samples.

Typical Standard Curve



Detection Range

0.33 – 27 ng/ml

Sensitivity

0.3 ng/ml