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Caffeine ELISA Kit

rev 02/21

(Catalog # E4558-100; 100 assays, Store kit at -20 °C)

I. Introduction:

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. **BioVision's Caffeine ELISA Kit** is a competitive-based ELISA that can quantify as low as 0.3 ng/ml caffeine in urine, saliva and serum samples within 90 minutes.

II. Applications:

- *In vitro* (high-throughput compatible) semi-quantitative determination of caffeine
- Detection Range: 0.33 – 27 ng/ml
- Sensitivity: 0.3 ng/ml

III. Sample Types:

Serum, urine and saliva

IV. Kit Contents:

Components	E4558-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4558-100-1
Standard	2 vials	Yellow	E4558-100-2
HRP Conjugate Stock	8 µl	Blue	E4558-100-3
Antibody	7 ml	NM/Red	E4558-100-4
TMB Substrate	10 ml	Amber	E4558-100-5
Stop Solution	10 ml	NM/Blue	E4558-100-6
Sample Diluent	20 ml	NM	E4558-100-7
Wash Buffer (10X)	50 ml	NM	E4558-100-8
Serum Solution	1.7 ml	Brown	E4558-100-9
Standard Buffer	25 ml	WM	E4558-100-10
Conjugate Buffer	7.5 ml	NM/Green	E4558-100-11
Plate Sealers	4	--	E4558-100-12

V. User Supplied Reagents and Equipment:

- 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

VI. Storage and Handling:

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.

VII. Reagent and Standard Preparation:

Bring all reagents to room temperature (RT) 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 RT 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.

Standards	S0	S1	S2	S3	S4	S5
Concentrations (ppb)	0	0.33	1	3	9	27

VIII. Sample 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

IX. Caffeine ELISA Assay Protocol:

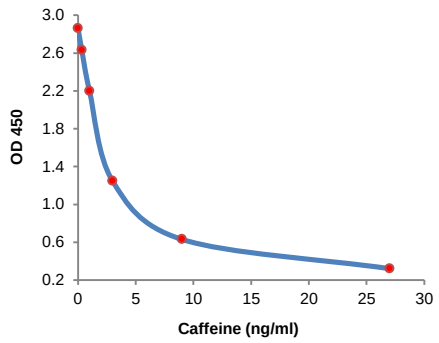
Notes: It is recommended that all standards and samples should be run at least in duplicate. Standard curves must be run each time as reference for sample quantification.

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 sec. 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.

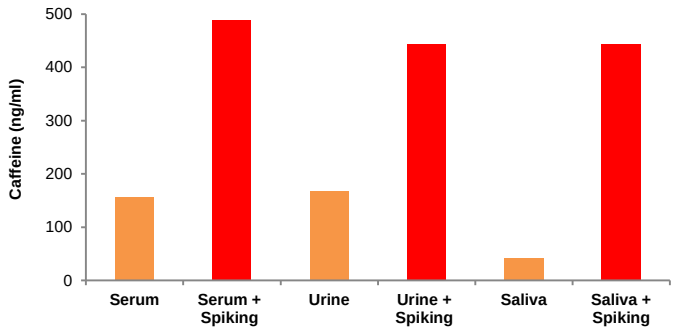
X. Calculation:

The Standard Curve is prepared by plotting OD at 450 nm vs. caffeine concentrations. The concentration of caffeine in each sample (ng/ml), which can be read from the calibration curve, is multiplied by the corresponding dilution factor.

A.



B.



Figures. **A.** Caffeine Standard Curve (*This standard curve is for demonstration only. A standard curve must be run with each assay*). **B.** Spike recovery experiment: Human serum, urine and saliva samples were assayed alone or with a spike to a final expected concentration of 500 ng/ml of caffeine (80-95% recovery of spike).

XI. RELATED PRODUCTS:

Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
Ampicillin ELISA Kit (Cat. No. E4350-100)
Enrofloxacin (ENR) ELISA Kit (Cat. No. E4277-100)
Quinolone ELISA Kit (Cat. No. E4530-100)
Folic Acid ELISA Kit (Cat. No. 4523-100)
Kanamycin ELISA Kit (Cat. No. K4210-100)
Cell Lysis Buffer (Cat. No. 1067-100)

FOR RESEARCH USE ONLY! Not to be used on humans.