# ab285229 – Caffeine ELISA Kit

For the *in vitro* (high-throughput compatible) semi-quantitative determination of caffeine.
For research use only - not intended for diagnostic use.
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

**For overview, typical data and additional information please visit:**
http://www.abcam.com/ab285229

## 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. Caffeine ELISA Kit (ab285229, E4558) 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.

## Applications

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

## Storage and Stability

On receipt entire assay kit should be stored at -20°C. Upon opening, use kit within 1 months.

## Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Caffeine-BSA Conjugate Coated Plate/ELISA Microplate | 8 x 12 strips | -20°C |
| Caffeine Standard/Standard | 2 vials | -20°C |
| Goat Anti-Mouse IgG HRP Conjugate I/HRP Conjugate Stock | 10 µl | -20°C |
| Caffeine Antibody/Antibody | 7 mL | -20°C |
| TMB Substrate I/TMB Substrate | 10 mL | -20°C |
| Stop Solution VIII/Stop Solution | 10 mL | -20°C |
| Sample Diluent IV/Sample Diluent | 20 mL | -20°C |
| 10X Wash Buffer II/Wash Buffer (10X) | 50 mL | -20°C |
| Serum Solution II/Serum Solution | 1.7 mL | -20°C |
| Standard Buffer II/Standard Buffer | 25 mL | -20°C |
| Conjugate Buffer I/Conjugate Buffer | 7.5 mL | -20°C |
| Microplate Sealing Film/Plate Sealers | 4 units | -20°C |



## Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 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
-

## Reagent Preparation

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

Caffeine Antibody/Antibody, TMB Substrate I/TMB Substrate, Stop Solution VIII/Stop Solution, Sample Diluent IV/Sample Diluent, Serum Solution II/Serum Solution, Standard Buffer II/Standard Buffer and Conjugate Buffer I/Conjugate Buffer: Ready to be used. After use, store them at 4 °C.

Goat Anti-Mouse IgG HRP Conjugate I/HRP Conjugate Stock: Spin briefly before opening the tube. Pipette 2 µL of Goat Anti-Mouse IgG HRP Conjugate I/HRP Conjugate Stock into Conjugate Buffer I/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.

10X Wash Buffer II/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 II/Wash Buffer by diluting 10 mL of Wash Buffer II/Wash Buffer with 90 mL deionized water. Concentrated and diluted Wash Buffer II/Wash Buffer can be stable at 4 °C for 3 months.

Caffeine Standard/Standard: Add 1.5 mL of Standard Buffer II/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 II/Standard Buffer) to prepare S4 to S1 standards sequentially. S0 contains Standard Buffer II/Standard Buffer only. Diluted standards can be stored at -20 °C for 2 weeks.

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

## Sample Preparation

Serum

1. Add 20 µL of Serum Solution II/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 IV/Sample Diluent. (For example, mix 5 µL of serum with 195 µL of Sample Diluent IV/Sample Diluent.)
5. Use 50 µL per well for the assay.

**Δ 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 IV/Sample Diluent. (For example, mix 5 µL of urine with 195 µL of Sample Diluent IV/Sample Diluent.)
3. Use 50 µL per well for the assay.

**Δ Note:** *Dilution factor: 40*

## Assay Protocol

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

1. Prepare all reagents, standards, and samples.
2. Add 50 µL of Standards or Samples per well. Add 50 µL of conjugate working solution and 50 µL of Caffeine Antibody/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 II/Wash Buffer and incubate for 30 sec. Remove 1X Wash Buffer II/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 I/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 I/TMB Substrate), add 50 µL of Stop Solution VIII/Stop Solution and gently tap the plate to ensure thorough mixing.
7. Measure OD at 450 nm for the standards and samples.

## 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.

## Technical Support


For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)