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.

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

Storage and Stability

On receipt entire assay kit should be stored at -20°C. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
ELISA Microplate	8 x 12 strips	-20°C
Standard	2 vials	-20°C
HRP Conjugate Stock	8 µl	-20°C
Antibody	7 mL	-20°C
TMB Substrate	10 mL	-20°C
Stop Solution	10 mL	-20°C
Sample Diluent	20 mL	-20°C
Wash Buffer (10X)	50 mL	-20°C
Serum Solution	1.7 mL	-20°C
Standard Buffer	25 mL	-20°C
Conjugate Buffer	7.5 mL	-20°C
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.

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
Concs (ppb)	0	0.33	1	3	9	27

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.

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. (For example, mix 5 µL of urine with 195 µL of 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 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.

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)

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