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ab242297
CPD ELISA Kit (cyclobutane pyrimidine dimers, DNA samples)

View CPD ELISA Kit (cyclobutane pyrimidine dimers, DNA samples) datasheet:
www.abcam.com/ab242297

For the quantitative measurement of CPD in cell or tissue samples.

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1. Overview

CPD ELISA Kit (ab242297) is developed for rapid detection and quantitation of CPD in any DNA samples.

The quantity of CPD in unknown sample is determined by comparing its absorbance with that of a known CPD-DNA standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

2. Protocol Summary

Prepare all reagents, samples and standards as instructed.

Add 50 µL standard or sample, then 50 µL DNA Binding Solution to wells of DNA High-Binding plate and incubate overnight at room temperature.

Washing steps with PBS. Then add 200 µL Assay Diluent to all wells and incubate for 1 hr at room temperature.

Add 100 µL diluted anti-CPD antibody to all wells and incubate for 1 hr at room temperature. Then washing steps with 1X Wash Buffer.

Add 100 µL diluted Secondary Antibody-HRP Conjugate per well and incubate for 1 hour. Wash as before with 1X Wash buffer.

Add 100 µL of warm Substrate Solution and incubate at room temperature for 2-30 minutes.

Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Read absorbance immediately on a microplate reader using 450 nm.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Materials Supplied, and Storage and Stability

- Store kit at +4°C immediately upon receipt and check below for storage for individual components
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
DNA High-Binding Plate	96 well	+4°C
DNA Binding Solution	6 mL	+4°C
Anti-CPD Antibody	20 µL	+4°C
Secondary Antibody, HRP Conjugate	50 µL	+4°C
Assay Diluent	50 mL	+4°C
10X Wash Buffer	100 mL	+4°C
Substrate Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
CPD-DNA Standard	100 µL	-20°C
Reduced DNA Standard	100 µL	-20°C

5. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at O.D. 450 nm (620 nm as optional reference wave length)

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 1X Wash Buffer:

6.1.1 Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.

6.2 Anti-CPD antibody:

6.2.1 Immediately before use dilute the Anti-CPD Antibody 1:1000 with Assay Diluent.

6.3 Secondary Antibody:

6.3.1 Immediately before use dilute the Secondary Antibody 1:1000 with Assay Diluent.

Δ Note: Do not store diluted solutions.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Convert CPD-DNA standard (25 µg/mL) and Reduced DNA (200 µg/mL) to single-stranded DNA by incubating the DNA at 95°C for 10 minutes and rapidly chilling on ice for 10 minutes.

Δ Note: Aliquot and store denatured DNA at -20°C. Repeat the above denaturation step every time you prepare the CPD-DNA standard.

7.2 Freshly prepare 4 µg/mL of CPD-DNA by diluting the denatured 25 µg/mL stock in cold TE Buffer. Example: Add 8 µL to 42 µL of cold TE Buffer.

7.3 Freshly prepare 4 µg/mL of Reduced DNA by diluting the denatured 200 µg/mL stock in cold TE Buffer. Example: Add 40 µL to 1.96 mL of cold TE Buffer.

7.4 Prepare a series of CPD-DNA standards according to the table below:

Standard #	4 µg/mL denatured CPD-DNA (µL)	4 µg/mL Denatured Reduced DNA (µL)	CPD-DNA (ng/mL)
1	10	390	100
2	200 of standard #1	200	50
3	200 of standard #2	200	25
4	200 of standard #3	200	12.5
5	200 of standard #4	200	6.25
6	200 of standard #5	200	3.13
7	200 of standard #6	200	1.56
8	0	200	0

8. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

8.1 Extract DNA from cell or tissue samples using a commercial DNA Extraction kit or other desired method.

8.2 Convert DNA sample to single-stranded DNA by incubating the sample at 95ºC for 10 minutes and rapidly chilling on ice for 10 minutes.

8.3 Dilute DNA samples to 4 µg/mL in cold TE Buffer.

Δ Note: Samples with high concentrations of CPD may be further diluted 2-4 fold in 4 µg/mL Reduced DNA. A titration may be performed to ensure the samples fall in the range of the standard curve

9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

9.1 Add 50 µL of unknown DNA samples or CPD-DNA standards to the wells of the DNA High- Binding plate.

9.2 Add 50 µL of DNA Binding Solution to each well. Mix well by pipetting and incubate at room temperature overnight on an orbital shaker. Each DNA sample including unknown and standard should be assayed in duplicate.

9.3 Remove the DNA solutions and wash twice with PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hour at room temperature.

9.4 Remove the Assay Diluent. Blot plate on paper towels to remove excess fluid.

9.5 Warm 100 µL of the diluted Anti-CPD Antibody to all wells and incubate for 1 hour at room temperature on an orbital shaker.

9.6 Wash 5 times with 250 µL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

9.7 Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 5 times according to step 9.6 above.

9.8 Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes

Δ Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9.9 Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).

9.10 Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

10. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

10.1 Average the duplicate reading for each standard, control and sample.

10.2 Subtract the mean value of the blank (Standard #8) from all standards, controls and sample readings. This is the corrected absorbance.

10.3 If significant, subtract the sample background control from sample readings.

10.4 Plot the corrected values for each standard as a function of the final concentration of CPD-DNA.

10.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

10.6 Apply the corrected sample OD reading to the standard curve to get CPD-DNA amount in the sample wells.

10.7 Concentration of CPD-DNA in ng/mL in the test samples is calculated as:

$$CPD - DNA\ concentration = \mathrm{B} * D$$

Where:

B = amount of CPD-DNA in the sample well calculated from standard curve in ng/mL

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

11. Typical Data

Typical standard curve - data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

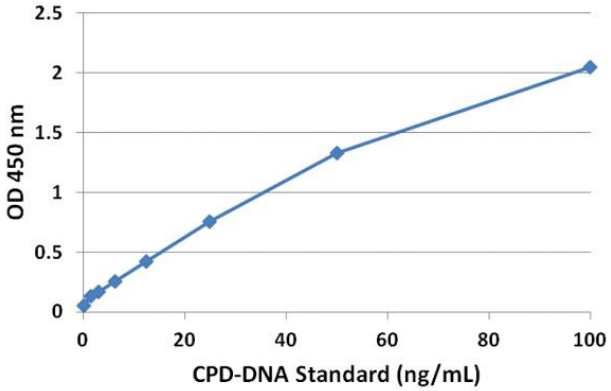

Figure 1. Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

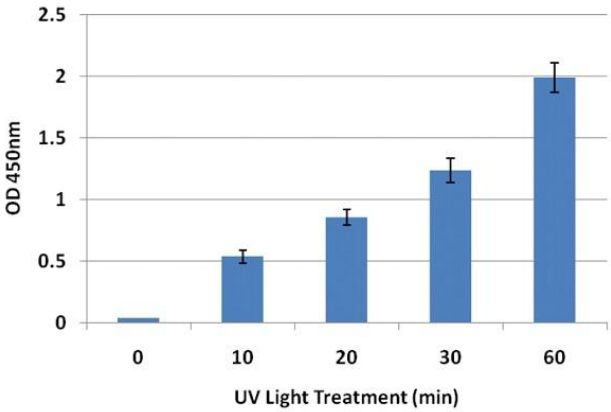

Figure 2. DNA Damage Induced by UV Light. 0.2 mg/mL Calf thymus DNA was exposed to UV light inside a cell culture hood for the time indicated. The CPD levels in denatured DNA samples were determined as described in the Assay Protocol.

12. Species and Cross Reactivity

This kit is not species specific and can be used with samples from any species.

Please contact our Technical Support team for more information.

13. Notes

Technical Support

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