Pyrimidine Dimer ELISA Kit

Pyrimidine Dimer ELISA Kit is an Enzyme Immunoassay kit for the quantification of Pyrimidine Dimer in Cell or Tissue genomic DNA

Catalog number: ARG83380

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

Pyrimidine dimers are molecular lesions formed from thymine or cytosine bases in DNA via photochemical reactions, commonly associated with direct DNA damage. Ultraviolet light (UV) induces the formation of covalent linkages between consecutive bases along the nucleotide chain in the vicinity of their carbon–carbon double bonds. The photo-coupled dimers are fluorescent. The dimerization reaction can also occur among pyrimidine bases in dsRNA—uracil or cytosine. Two common UV products are cyclobutane pyrimidine dimers and 6–4 photoproducts. These premutagenic lesions alter the structure of the DNA helix and cause non-canonical base pairing. Specifically, adjacent thymines or cytosines in DNA will form a cyclobutane ring when joined together and cause a distortion in the DNA. This distortion prevents replication or transcription machinery beyond the site of the dimerization. Up to 50–100 such reactions per second might occur in a skin cell during exposure to sunlight, but are usually corrected within seconds by photolyase reactivation or nucleotide excision repair. In humans, the most common form of DNA repair is nucleotide excision repair. In contrast, organisms such as bacteria can counterintuitively harvest energy from the sun to fix DNA damage from pyrimidine dimers via photolyase activity. If these lesions are not fixed, polymerase machinery may misread or add in the incorrect nucleotide to the strand. If the damage to the DNA is overwhelming, mutations can arise within the genome of an organism and may lead to the production of cancer cells. Uncorrected lesions can inhibit polymerases, cause misreading during transcription or replication, or lead to arrest of replication. It causes sunburn and it triggers the production of melanin. Pyrimidine dimers are the primary cause of melanomas in humans.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. Standards or samples are pipetted into the wells and any Pyrimidine Dimer present is bound by the DNA. After washing away any unbound substances, a biotin-conjugated antibody specific for Pyrimidine Dimer is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Pyrimidine Dimer bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ± 2nm. The concentration of Pyrimidine Dimer in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Pyrimidine Dimer Standard and Blank (Reduced DNA) at ≤ -20°C, Store other component at 2-8°C at all times.

Component	Quantity	Storage information
DNA Microplate	8 x 12 strips	4°C.
DNA Binding Solution	6 mL	4°C.
Pyrimidine Dimer Standard	1 vial (100 µL; 25 µg/mL)	-20°C
Blank (Reduced DNA)	1 vial (100 µL; 0.2 mg/mL)	-20°C
1000X Pyrimidine Dimer Antibody	1 vial (20 µL)	4°C
1000X HRP-Streptavidin Solution	1 vial (50 µL)	4°C
Diluent Buffer	50 mL	4°C
10X Wash Buffer	100 mL	4°C
TMB Substrate	12 mL	4°C (Protect from light)
Stop Solution	12 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- TE buffer
- Automated microplate washer (optional)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store Pyrimidine Dimer Standard and Blank (Reduced DNA) at ≤ -20°C, Store other component at 2-8°C at all times. Use the kit before expiration date.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

DNA: After Extract DNA from cell or tissue, incubating the sample at 95°C for 10 minutes and rapidly put on ice for 10 minutes to convert DNA sample to single-stranded DNA, and Dilute DNA samples to 4 µg/mL in cold TE Buffer

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute **10X** Wash Buffer into distilled water to yield 1X Wash Buffer.
- **1X Pyrimidine Dimer Antibody:** 10 minutes before use, dilute **1000X** Pyrimidine Dimer Antibody into Diluent Buffer to yield **1X** Pyrimidine Dimer Antibody.
- **1X HRP-Streptavidin Solution:** 10 minutes before use, dilute **1000X** HRP-Streptavidin Solution into Diluent Buffer to yield **1X** HRP-Streptavidin Solution. Keep diluted HRP-Streptavidin Solution in dark before use.
- **Blank (Reduced DNA)**: Convert Blank to single-stranded DNA as sample. Dilute Blank into cold TE Buffer at 1:6.25 dilution (ex. add 8 µL Blank into 42 µL cold TE Buffer).
- **Standard:** Convert Standard to single-stranded DNA as sample. The Diluent Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Diluent Buffer. Diluted the standard as below:

Standard tube	Pyrimidine Dimer (ng/mL)	Diluent Buffer (ng/mL)	Standard (ng/mL)
S1	100	490	10 (50 µM Stock)
S2	50	250	250 of S1
S3	25	250	250 of S2
S4	12.5	250	250 of S3
S5	6.25	250	250 of S4
S6	3.125	250	250 of S5
S7	1.562	250	250 of S6
S0	0	250	0

Note: Repeat the denaturation step every time prepare the Blank / standard.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add **50 µL** of **Samples, Standard** into respective wells of the 96-well plate.
2. Add **50 µL** of **DNA Binding Solution** into each wells of the 96-well plate.
3. Cover the plate and incubate for **overnight** at **4°C**.
4. Aspirate each well and wash, repeating the process 1 time for a **total 2 washes.** Wash by filling each well with **PBS (250 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **200 µL** of **Diluent Buffer** to each well.
6. Cover the plate and incubate for **1 hour** at **RT** for block.
7. Add **100 µL** of **1X Pyrimidine Dimer Antibody** to each well.
8. Cover the plate and incubate for **1 hour** at **RT**.
9. Aspirate each well and wash, repeating the process 4 time for a **total 5 washes.** Wash by filling each well with **1X wash buffer (250 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
10. Add **100 µL** of **1X HRP-Streptavidin Solution** to each well.
11. Cover the plate and incubate for **1 hour** at **room temperature** in the dark.
12. Aspirate each well and **wash** plate **as step 9**.
13. Add **100 µL** of **TMB Substrate** in each well.

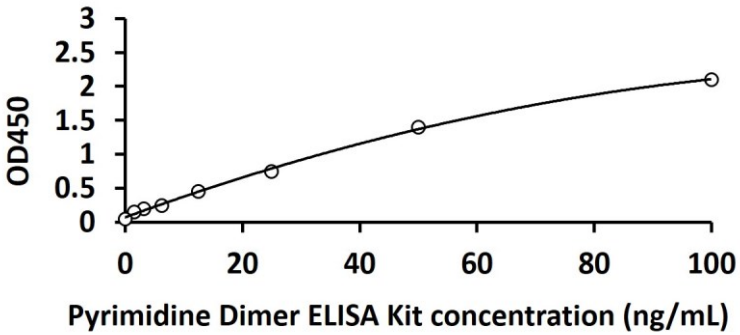
14. Incubate for **5-30 mins** at **room temperature** in the dark.
15. Add **100 µL** of **Stop Solution** to each well to stop the reaction.
16. Read the absorbance with a plate reader at **O.D. 450 nm.** It is recommended reading the absorbance within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Pyrimidine Dimer ELISA Kit. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

0.8 ng/mL

Assay Range

1.56- 100 ng/mL