DATA SHEET

ELISA Kit for 8-Hydroxydeoxyguanosine (8-OHdG)

Organism species	General
Product No.	CEA660Ge
Sample type	Serum, plasma and other biological fluids.
Format	96-well strip plate
Assay length	2.5 hours
Detection range	49.38-4000pg/mL The standard curve concentrations used for the ELISA's were 4000pg/mL, 1333.33pg/mL, 444.44pg/mL, 148.15pg/mL, 49.38pg/mL
Sensitivity	The minimum detectable dose of this kit is typically less than 18.5pg/mL.

Specificity

This assay has high sensitivity and excellent specificity for detection of 8-Hydroxydeoxyguanosine (8-OHdG).
No significant cross-reactivity or interference between 8-Hydroxydeoxyguanosine (8-OHdG) and analogues was observed.

Recovery

Matrices listed below were spiked with certain level of recombinant 8-Hydroxydeoxyguanosine (8-OHdG) and the recovery rates were calculated by comparing the measured value to the expected amount of 8-Hydroxydeoxyguanosine (8-OHdG) in samples.

Matrix	Recovery range (%)	Average(%)
serum(n=5)	80-90	87
EDTA plasma(n=5)	98-105	101
heparin plasma(n=5)	89-103	101

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level 8-Hydroxydeoxyguanosine (8-OHdG) were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level 8-Hydroxydeoxyguanosine (8-OHdG) were tested on 3 different plates, 8 replicates in each plate.
CV(%) = SD/meanX100
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of 8-Hydroxydeoxyguanosine (8-OHdG) and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	81-88%	98-105%	78-98%	84-91%
EDTA plasma(n=5)	95-103%	96-103%	87-94%	89-97%
heparin plasma(n=5)	80-92%	79-101%	83-92%	80-91%

Stability

The stability of kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage condition.
To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator

temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

Reagents and materials provided

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120µL	Assay Diluent A	1×12mL
Detection Reagent B	1×120µL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

Assay procedure summary

1. Prepare all reagents, samples and standards;
2. Add 100µL standard or sample to each well. Incubate 2 hours at 37^{o}C;
3. Aspirate and add 100µL prepared Detection Reagent A. Incubate 1 hour at 37^{o}C;
4. Aspirate and wash 3 times;
5. Add 100µL prepared Detection Reagent B. Incubate 30 minutes at 37^{o}C;
6. Aspirate and wash 5 times;
7. Add 90µL Substrate Solution. Incubate 15-25 minutes at 37^{o}C;

8. Add 50µL Stop Solution. Read at 450nm immediately.

Test principle

The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to 8-Hydroxydeoxyguanosine (8-OHdG). Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to 8-Hydroxydeoxyguanosine (8-OHdG). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain 8-Hydroxydeoxyguanosine (8-OHdG), biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of 8-Hydroxydeoxyguanosine (8-OHdG) in the samples is then determined by comparing the O.D. of the samples to the standard curve.