Application of New 8-OHdG Check to auto analyzer.
(code: KOG-200S/E)

1) Application example.

Kyowa Medex Co., Ltd. Model AP-960

2) Settings for New 8-OHdG Check application in AP-960.

A) Premix:

Aspirate 50 micro L of primary antibody, then aspirate 50 micro L of samples into the same nozzle (disposable pipettes). Then dispense 100 micro L of mixture to the well.

There may be no problem in the procedure "Aspirate 60 micro L of primary antibody and dispense 50 micro L, and aspirate 60 micro L of samples/ standards and dispense 50 micro L".

B) Plate mixing:

Horizontally shake the micro plate at 16 minutes and 35 minutes after primary reaction have started.

C) Washing buffer volume:

Wash with 350 micro L of washing buffer for 3 times.

D) Chromatic reaction:

The chromatic reaction should be performed at 37 degree C for 10 minutes.

3) Important points.

A) Temperature control.

8-OHdG Check ELISA system in temperature sensitive. Although AP-960 is equipped with heating unit and optional cooling unit, but the cooling unit should not be used. Because the temperature of micro plate is not homogeneous enough when we tried to use cooling unit. Therefore we always control the room temperature at 25 degree C, and use only heating unit.

B) Washing procedure.

The volume of washing solution is changed from 250 micro L to 350 micro L.

Wash for 3 times. 8-OHdG ELISA is very sensitive to residue and well-to-well contamination of primary antibody. In case if the blank wells turn blue, the micro plate to paper towels to remove the residual solution. But please take care not to dry them up.

C) Well to well contamination of primary antibody.

To prevent well-to-well contamination, 96-pin type washing nozzle is ideal.

4) Preparation of urine and QC samples.

Urine samples should be thawed out by incubation at 37 degree C for 1 hour or 4 degree C for over night. Please remove insoluble materials just before the application to ELISA.

5) Calibration curves.

Spline algorithm with log (8-OHdG conc.) and linear (absorbance) is suitable. Almost any types of calibration algorithm using semi-log plot may be applicable.

	Absorbance at 450 nm		
	Day 1	Day 2	Day 3
0.5 ng/mL	1.802	1.954	1.930
2.0 ng/mL	1.570	1.747	1.733
8.0 ng/mL	1.139	1.334	1.312
20 ng/mL	0.721	0.871	0.852
80 ng/mL	0.292	0.340	0.348
200 ng/mL	0.177	0.199	0.202
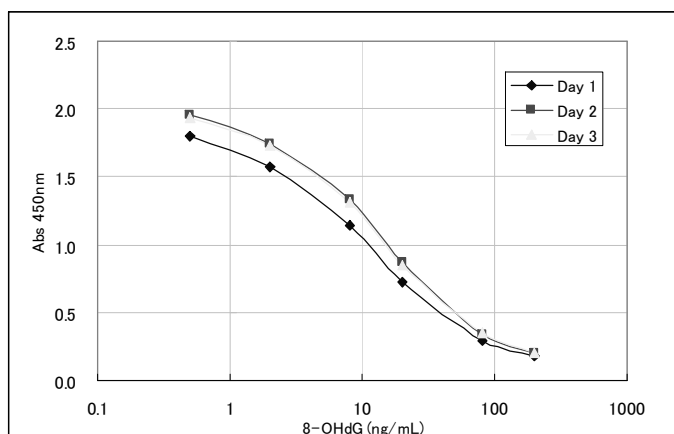
6) Intra-assay variation of 8-OHdG ELISA by AP-960.

	8-OHdG (ng/mL)									mean	S.D.	C.V.
Urine 1	4.1	4.5	5.2	4.3	5.6	6.2	5.9	4.8	4.2	5.0	0.8	
Urine 2	4.4	4.6	4.7	4.8	5.0	4.8	7.0	10.2	9.5	6.1	2.3	
Urine 3	12.1	14.8	12.8	13.6	14.3	15.4	14.8	16.7	18.7	14.8	2.0	14%
Urine 4	12.4	12.9	14.1	15.2	14.5	16.1	17.8	17.4	16.0	15.2	1.9	12%
Urine 5	28.3	35.6	34.3	35.4	36.8	37.3	36.2	34.8	28.3	34.1	3.4	10%
Urine 6	37.8	40.1	35.7	37.8	37.6	39.0	39.3	45.2	40.2	39.2	2.7	7%

	Absorbance at 450 nm								
Urine 1	1.632	1.601	1.551	1.614	1.521	1.480	1.499	1.582	1.632
Urine 2	1.604	1.590	1.588	1.577	1.567	1.580	1.433	1.250	1.287
Urine 3	1.158	1.051	1.131	1.098	1.067	1.030	1.051	0.988	0.927
Urine 4	1.148	1.124	1.077	1.036	1.061	1.005	0.952	0.963	1.008
Urine 5	0.719	0.616	0.634	0.618	0.601	0.596	0.609	0.626	0.720
Urine 6	0.590	0.566	0.614	0.590	0.593	0.577	0.574	0.520	0.565

7) Inter-assay variation of 8-OHdG ELISA by AP-960.

	8-OHdG value of QC sample at succesive 10 days. (ng/mL)										mean	S.D.	C.V.
	7-Feb	8-Feb	9-Feb	10-Feb	11-Feb	12-Feb	1-Mar	2-Mar	3-Mar	4-Mar			
QC urine 1	4.2	3.7	4.2	4.1	4.0	3.9	4.1	2.3	2.1	2.9	3.6	0.8	
QC urine 2	9.4	10.1	9.9	9.5	8.4	7.9	9.2	9.3	9.7	10.6	9.4	0.8	8%
QC urine 3	25.7	29.1	25.3	25.2	24.6	23.3	26.6	27.1	30.3	28.7	26.6	2.2	8%