



# **oxidized DNA**

**(8-hydroxy-2'-deoxyguanosine, 8-OHdG)**  
**ELISA**

**Serum Application**

**Supplementary to instruction for use**

EK-ODG

96 tests

For Research Use Only (RUO)

Revision date: 2009-11-20

### INTENDED USE

The BÜHLMANN oxidized DNA ELISA kit is designed for the quantitative determination of 8-hydroxy-2'-deoxyguanosine in human serum after filtration.

### MATERIALS REQUIRED BUT NOT PROVIDED

- Filtration Devices Vivaspin 500, Sartorius Stedim Biotech MWCO: 10.000 PES (polyethersulfone membrane); Code.No.: VS0101 (25 pieces), VS0102 (100 pieces). **Carrier required:** 2.2 ml/11 mm d.
- PBS (Phosphate buffered saline)
- Pipettes for sample delivery and removal. For maximum recovery use a thin gel loader typ.
- Centrifuge with swing bucket of fixed angle (minimum 25°) rotor e.g. Labnet Spectrafuge 24 D.
- Microtiter Plate reader for measurement of absorbance at 450 nm.

### SAMPLE PREPARATION

- Pipett 300 µl serum into the concentrator of the filtration device.
- Centrifuge for 15 min at 15'000 x g at 18-28 °C.
- Collect the filtrate and use it directly for the immunoassay. Freeze the samples at -20 °C, if not used immediately.

The effect of lipemic, icteric and hemolytic samples has not been tested.

### STANDARD DILUTION & ASSAY PROCEDURE

**Calibrator concentration:** The concentration of stock standard is 1 µg/ml. However, the nominal concentration printed on the label is 10 µg/ml, taking into account a 1:10 dilution of urinary samples (main application).

#### Calibrator dilution:

Reconstitute the calibrator and prepare serial dilutions of the Calibrator as follows:

- Label tubes S1 to S5 and pipet 930 µl of Incubation buffer (IB) into tube S1 and 300 µl IB into tubes S2 to S5.
- Pipet 30 µl of reconstituted Calibrator (1 µg/ml) into tube S1 and vortex.
- Transfer 200 µl from S1 to S2, vortex. Transfer 200 µl from S2 to S3, vortex. Continue to transfer 200 µl from each tube until dilution series is completed. The corresponding concentrations of 8-OHdG will be:

**S1 31.3 ng/ml**

**S4 2.0 ng/ml**

**S2 12.5 ng/ml**

**S5 0.8 ng/ml**

**S3 5.0 ng/ml**

A typical standard curve is presented in Table 1.

The diluted Calibrators are stable for 8 hours at 2-8°C. Prepare new dilutions each time a new assay is performed. Refer to calibrator dilution and assay procedure described in page 3/8 of the EK-ODG instruction for use. The established control values have been multiplied by 10 for the urin application. Please divide the mean value and the ranges by 10, if you want to use them in the serum application.

### REFERENCE RANGE

A reference range (Min.-Max.) of 2.5 bis 4.8 ng/ml 8-OHdG has been established with 20 blood donors.

The arithmetic mean was established to be at **3.8 ng/ml**.

### PERFORMANCE CHARACTERISTICS

**Intra-Assay Precision: 3.5 - 12 %.** Two sera were spiked with 1, 2, 4, and 10 ng/ml 8-OHdG. The intra-assay precision was calculated from 16 replicates each in a single run according to the assay procedure. The values are presented in Table 2.

**Functional Sensitivity: < 2 ng/ml.** The functional sensitivity was deduced from the dilution experiments to be at < 2 ng/ml.

**Dilution Linearity: 92.5-131 %.** Three serum samples were spiked with 8-OHdG, diluted serially with PBS 1:2 to 1:16, and subsequently assayed according to the assay procedure. The results are presented in Table 3.

**Spiking Recovery: 92.1-108.1 %.** Two serum samples were spiked with 8-OHdG at a concentration of 1, 2, 4, 10 ng/ml. The samples were measured before and after spiking according to the assay procedure. The results are presented in Table 2.

### APPENDIX I

#### TABLES/ TABELLEN/ TABLES/ TABELLE/ TABLAS

Table 1: Example of Results

	Absorb. [OD]	CV [%]	B/B <sub>0</sub> [%]	Conc. [ng/ml]
Blank	0.054			
B 0 Avg.	2.499 2.417 2.458	2.3	101.7 98.3 100.0	0.0
Cal S1	2.312		94.1	
Cal S1	2.258		91.8	
Cal A Avg.	2.285	1.7	93.0	0.8
Cal S2	1.875		76.3	
Cal S2	1.775		72.2	
Cal B Avg.	1.825	3.9	74.3	2.0
Cal S3	1.057		43.0	
Cal S3	1.015		41.3	
Cal C Avg.	1.036	2.9	42.1	5.0
Cal S4	0.450		18.3	
Cal S4	0.430		17.5	
Cal D Avg.	0.440	3.1	17.9	12.5
Cal S5	0.145		5.9	
Cal S5	0.138		5.6	
Cal E Avg.	0.142	3.3	5.8	31.3
Control low	1.495			
Control low	1.320			
Control low Avg.	1.408	8.8		3.3
Control high	0.300			
Control high	0.275			
Control high Avg.	0.288	6.0		17.4

Table 2: Spiking Recovery /Intra-Assay Precision

Sample	Spiked with 8-OHdG	Mean [ng/ml]	SD [ng/ml]	RSD [%]	O/E [%]
1		3.4	0.41	12	-
	1	4.2	0.35	8.2	96.1
	2	5.4	0.37	6.9	99.8
	4	7.3	0.32	4.3	98.9
	10	12.3	0.44	3.5	92.0
Mean				7.0	96.7
2		2.3	0.28	12	
	1	3.6	0.25	7	106
	2	4.7	0.43	9.3	108
	4	6.6	0.4	6.1	104
	10	11.8	0.53	4.5	95.5
Mean				7.8	103

**Table 3: Dilution Linearity/Parallelism**

Sample	Dilution	Observed [ng/ml]	Expected [ng/ml]	O/E [%]
S1	1:1	35	-	-
	1:2	18.6	18.6	100
	1:4	8.6	9.3	92.5
	1:8	4.7	4.7	101.1
	1:16	2.4	2.3	104
	Mean			
S2	1:1	33.4	-	-
	1:2	17.6	17.6	100
	1:4	9.4	8.8	107
	1:8	5.5	4.4	125
	1:16	2.9	2.2	131
	Mean			
S3	1:1	32.4	-	-
	1:2	17.5	17.5	100
	1:4	8.9	8.7	102
	1:8	4.7	4.4	108
	1:16	2.52	2.2	115
	Mean			
<b>Mean</b>				<b>107</b>

**Table description:** cf. "Results" (page 2), "Performance Characteristics" (page2).

Oxidised DNA ELISA

**Sample preparation**

Pipett 300 µl serum into the filtration device, Vivaspin 500



*Centrifuge for 15 min. at 15'000 x g at 18-28 °C*

The filtrate can be used directly in the Immunoassay

**Immunoassay**

Precoated Microtiter Plate



*wash 2 x*

50 µl Calibrators, Controls, Samples  
+  
50 µl 8OHdG-Biotin-Conjugate  
+  
50 µl 8OHdG-Antiserum



*incubate 5±1 minutes at 18-25°C on a plate rotator*



*incubate 2 hours ± 5 min at 2-8°C*



*wash 3 x*

add 100 µl Enzyme Label



*incubate 30±1 minutes at 18-25°C on a plate rotator*



*wash 3 x*

add 100 µl TMB Substrate



*incubate 15±1 minutes at 18-25°C on a plate rotator*

add 100 µl Stop Solution

➔ Read absorbance at 450 nm (within 30 minutes)

ASSAY TIME: 3 H 05 MIN