



# Oxidized DNA ELISA

Procedure

EK-ODG

A Commitment to Diagnostics

## Pre-Analytics

Sample specimen and volume: Less than 100 µl urine; 300 µl serum

Sample storage: at 2-8° C up to 7 days  
at -20°C at least 4 months

## Procedural Notes

Stability of diluted calibrators: 8 hours.  
Dilute calibrators freshly from the stock calibrator every time the assay is performed.

Constant incubation temperature is essential to ensure reproducibility of the results.

## Kit Components

Reagents	EK-ODG
Mikrotiter plate (wells)	96
Incubation Buffer	1 x 50 ml
Calibrator	1 x lyoph.
Controls - Low, High	2 x lyoph.
Enzyme Label	1 x 11 ml
Biotin Conjugate	1 x 5.5 ml
Substrate (TMB)	1 x 11 ml
Stop Solution (H <sub>2</sub> SO <sub>4</sub> )	1 x 11 ml
Wash Buffer Concentrate	1 x 100 ml

## ELISA Procedure

### Precoated microtiter plate

↓ ↻ wash 2x

### 50 µl calibrators, controls and urine-samples (1:10)

add 50 µl of Biotin conjugate

add 50 µl of 80HdG-antiserum

125' ↓ ↻ incubate 5 minutes at 18-25°C on a plate shaker

↓ ↻ incubate 2 h at 2-8°C

↓ ↻ wash 3 x

add 100 µl of Enzyme label

30' ↓ ↻ incubate 30 minutes at 18-25°C on a plate shaker

↓ ↻ wash 3 x

add 100 µl of TMB substrate

15' ↓ ↻ incubate 15 min at 18-25°C on a plate shaker

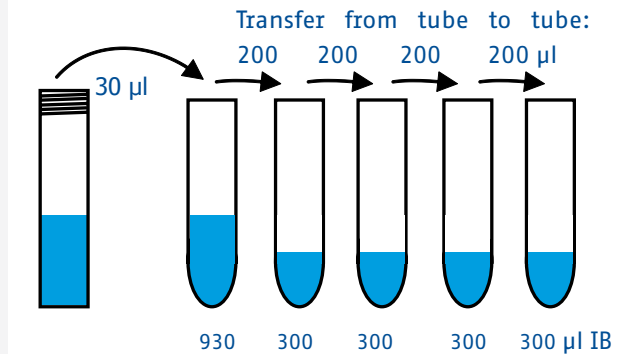
add 100 µl of Stop solution

→ Read at 450 nm

## Preparation of calibrators

Reconstitute the stock calibrator. Prepare serial dilutions of the calibrator as follows:

Reconstitute with 1 ml Incubation buffer (IB)



Blank	S1	S2	S3	S4	S4
10 µg/ml	312.5 ng/ml	125 ng/ml	50 ng/ml	20 ng/ml	8 ng/ml

Time to Result: 170 minutes





# Oxidized DNA ELISA

## Characteristics

## EK-ODG

A Commitment to Diagnostics

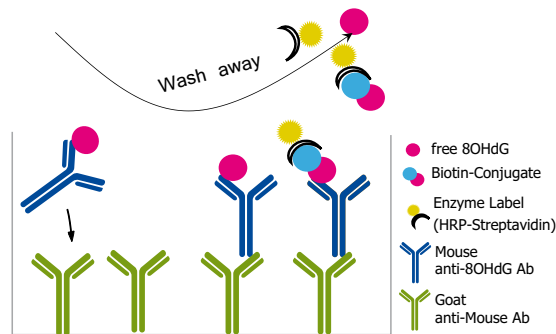
### Intended Use

The BÜHLMANN Oxidized DNA ELISA kit has been designed for the direct and quantitative determination of 8-hydroxy-2-deoxyguanosine (8-OHdG) in urine. A serum application is available for download on the website: [www.buhlmannlabs.ch](http://www.buhlmannlabs.ch)

For Research Use Only

### Principle of the Assay

#### Competitive Immunoassay



### Number of Tests

96 Well Microtiter Plate equivalent to

38 Patient samples\* in 1 run.

1 Blank\* (reconstituted Calibrator - 10 µg/ml)

1 B0 Zero Calibrator\* (Incubation buffer)

5 Calibrators\* (S5-S1)

2 Controls\* (low and high)

\* duplicates

### Assay Performance Data

**Intra-assay precision** 6.8 %  
n=6; range: 15.3-253.0 ng/ml  
20 duplicates; CV range: 2.8-10.6 %

**Inter-assay precision** 11.7 %  
n=6; range: 15.4-180.0 ng/ml  
20 repetitions; CV range: 6.7-17.0 %

**Dilution linearity** 96.5 %  
n=5; diluted 1:10 to 1:320  
recovery range: 84.0-113.2 %

**Spiking recovery** 102.5 %  
n=3; spiked 10-200 ng/ml  
recovery range: 79.3-124.9 %

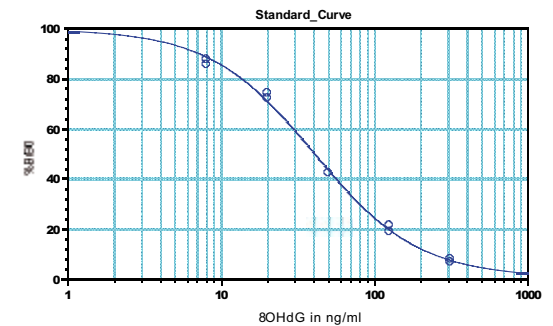
**Analytical sensitivity** <5 ng/ml  
Concentration at the mean of the Zero Calibrator (B0) minus 2 SD.

**Functional sensitivity** <13.5 µg/ml  
Intra-assay Precision <15 %CV

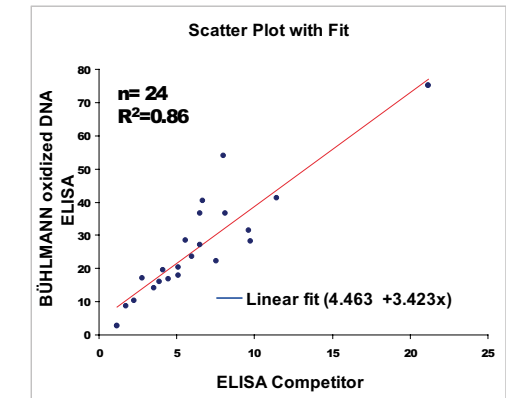
#### Specificity (50 % binding)

8-Hydroxydeoxyguanosine	100 %
8-Hydroxyguanosine	20 %
8-Hydroxyguanine	17 %
2'-Deoxyguanosine monohydrate	<0.01 %
6-Methyl-2'-deoxyguanosine	<0.01 %
8-Hydroxy-2'-deoxyadenosine	<0.01 %
8-Hydroxy-2'-deoxyadenosine	<0.01 %
2'-deoxycytidine	<0.01 %
Thymidine	<0.01 %
2'-deoxyuridine	<0.01 %
Guanosine	<0.01 %
8-mercaptopguanosine	<0.01 %
Guanine	<0.01 %
8-Bromoguanosine	<0.01 %
Creatine	<0.01 %
7-methylguanosine	<0.01 %
Creatinine	<0.01 %
Urea	<0.01 %

### Typical Standard Curve



### Method Comparison Bühlmann vs. Competitor



Product Code:  
EK-ODG

96 wells



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