DNA Damage Competitive ELISA Kit

Catalog Number EIADNAD (96 tests), EIADNADX10 (10 x 96 tests)

Rev 2.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The DNA Damage ELISA Kit is a solid-phase competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of DNA damage in serum, EDTA and heparin plasma, saliva, urine, digested DNA, fecal extracts, and tissue culture media. The assay recognizes DNA damage independent of species.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity (96 tests)	Quantity (10 x 96 tests)
8-Hydroxy-2'-deoxyguanosine Standard; 160 ng/mL DNA damage in a special stabilizing solution	70 µL	10 x 70 µL
Assay Buffer Concentrate (5X)	28 mL	10 x 28 mL
Antibody Coated Wells, 96-well strip-well plate coated with goat anti-rabbit IgG	1 plate	10 plates
8-Hydroxy-2'-deoxyguanosine Antibody	3 mL	10 x 3 mL
8-Hydroxyguanosine Conjugate	3 mL	10 x 3 mL
Wash Buffer Concentrate (20X)	30 mL	2 x 125 mL
TMB (Tetramethylbenzidine) Substrate	11 mL	10 x 11 mL
Stop Solution; contains 1 M HCl, CAUSTIC	5 mL	1 x 50 mL
Plate Sealer	1	10

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm (preferably with correction between 570 nm and 590 nm)
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.
- Solutions containing sodium azide will inhibit the activity of the peroxidase conjugate. Ensure that there is no contamination of labware or the plate washer with azide containing solutions.

Prepare 1X Wash Buffer

1. Dilute 15 mL of Wash Solution Concentrate (20X) with 285 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 3 months.

Prepare 1X Assay Buffer

1. Dilute 14 mL of Assay Buffer (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 4°C for 3 months.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge, or filter sample prior to analysis.

Prepare samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

Use all samples within **2 hours** of dilution, or store at –20°C or lower until ready to perform assay.

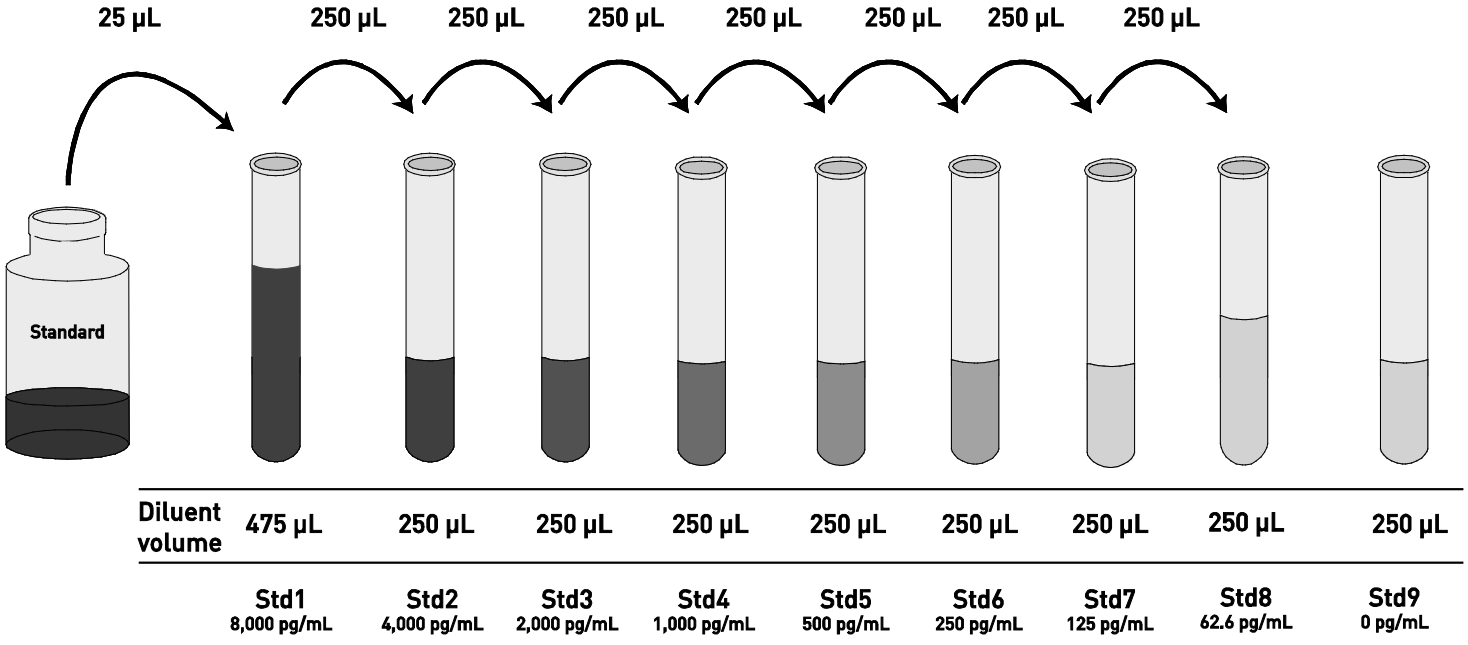
Sample type	Procedure
Serum and plasma	Dilute samples ≥1:8 with 1X Assay Buffer prior to running the assay.
DNA	See detailed extraction protocol on the product page at **thermofisher.com**
Saliva	Dilute saliva samples ≥ 1:2 with diluted 1X Assay Buffer prior to running the assay.
Urine	Dilute samples ≥1:4 with 1X Assay Buffer. **Note**: A Urinary Creatinine Detection Kit (Cat. no. EIACUN) is available for measuring urine creatinine for normalization of DNA damage in a random urine specimens.
Dried feces	See detailed extraction protocol on the product page at **thermofisher.com** **Note**: The ethanol concentration in the final diluted Assay Buffer dilution added to the well should be ≤2.5%.
Tissue culture media	Perform sample dilutions with the corresponding tissue culture medium.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Instructions are for diluting standards from 8,000 to 62.6 pg/mL, but a curve can be obtained using a range of 4,000 to 62.6 pg/mL. Choose the range that fits your sample concentrations most appropriately.

1. Add 25 µL 8-Hydroxy-2′-deoxyguanosine Standard to one tube containing 475 µL 1X Assay Buffer and label as 8,000 pg/mL 8-Hydroxy-2′-deoxyguanosine.
2. Add 250 µL 1X Assay Buffer to each of 8 tubes labeled as follows: 4,000; 2,000; 1,000; 500; 250; 125; 62.6; and 0 pg/mL 8-Hydroxy-2′-deoxyguanosine.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.

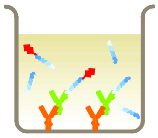


Perform ELISA (Total assay time: 2.5 hours)

IMPORTANT! Perform a standard curve with each assay.

Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

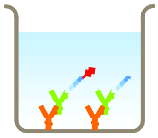
Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store desiccated at 2°C to 8°C for future use. The silica pack in the bag keeps the plate dry, and turns from blue to pink if the bag is not properly sealed.



Bind antigen

a. Add 50 µL of standards or samples (see "Prepare samples" on page 2) to the appropriate wells.
b. Add 75 µL of 1X Assay Buffer into wells for detecting non-specific binding (NSB).
c. Add 25 µL of 8-Hydroxy-2′-deoxyguanosine Conjugate to each well.
d. Add 25 µL of 8-Hydroxy-2′-deoxyguanosine Antibody to each well except NSB wells.
e. Tap the side of the plate to mix. Cover the plate with plate sealer and incubate for 2 hours at room temperature with shaking.

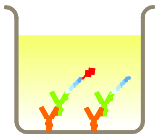
   Note: If the plate is not shaken the bound of the signals will be ~20% lower.
f. Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.



Add chromogen

a. Add 100 µL TMB Substrate to each well. The substrate solution will begin to turn blue.
b. Incubate for 30 minutes at room temperature without shaking.

   Note: TMB should not touch aluminum foil or other metals.



Add stop solution

Add 50 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 10 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four-parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns, and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals lower than that of the highest standard and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–8,000 pg/mL 8-Hydroxy-2′-deoxyguanosine.

Standard 8-Hydroxy-2′-deoxyguanosine (pg/mL)	Optical Density (450 nm)*
8,000	0.264
4,000	0.367
2,000	0.558
1,000	0.790
500	1.038
250	1.212
125	1.348
62.5	1.441
0	1.534

Note: The NSB gave a Mean OD value of 0.119.

Intra-assay precision

Samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	423.6	995.7	1,187
%CV	11.7	8.2	7.1

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed in duplicates in 19 assay runs by five operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	404.9	887.8	1,121
%CV	13.4	8.3	8.1

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Ten human serum samples were tested in the assay. Neat sample values ranged from approximately 9,300 to over 20,300 pg/mL with an average of 14,584 pg/mL.

Ten human EDTA plasma samples were tested in the assay. Neat sample values ranged from approximately 5,735 to almost 12,000 pg/mL with an average of 8,183 pg/mL.

Two human saliva samples were tested in the assay. Neat sample values were 3,580 and 7,190 pg/mL with an average of 5,386 pg/mL.

Thirteen human urine samples were tested in the assay. Neat sample values ranged from 26.0 to over 212 ng/mL with an average of 92.1 ng/mL.

Two digested DNA samples were tested in the assay. Neat sample values were 46.6 and 78.5 pg 8-HOdG/µg DNA with an average of 62.5 pg 8-HOdG/µg DNA.

Four mammalian fecal extracts were tested in the assay. Neat sample values ranged from 121.6 to 527.1 pg/mg dry fecal weight with an average of 315.9 pg/mg dry fecal weight.

Specificity

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	Cross-reactivity %
8-Hydroxy-2'-deoxyguanosine	100
8-Hydroxyguanosine	27.32
8-Hydroxyguanine	9.50

Sensitivity

The analytical sensitivity is 50.9 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 18 times, and calculating the corresponding concentration.

Recovery

Recovery in urine was determined by taking two samples, one with a low diluted 8-hydroxy-2'-deoxyguanosine level of 793.4 pg/mL and one with a higher diluted level of 5,238 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used. Linearity in serum and EDTA plasma were determined in a similar manner.

Urine

High Sample %	Low Sample %	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80	20	4,350	4,351	100.0
60	40	3,460	3,375	97.5
40	60	2,571	2,647	102.9
20	80	1,682	1,596	94.8
			Mean Recovery	**98.8%**

Serum

High Sample %	Low Sample %	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80	20	1,311	1,338	102.0
60	40	1,128	1,223	108.4
40	60	945.8	968.4	102.4
20	80	763.2	736.7	96.5
			Mean Recovery	**102.3%**

EDTA Plasma

High Sample %	Low Sample %	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80	20	2,076	2,065	99.5
60	40	1,772	1,709	96.4
40	60	1,468	1,414	96.3
20	80	1,164	1,148	98.6
			Mean Recovery	**97.7%**

Limited product warranty

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Catalog Number

Batch code


Temperature limitation


Use by


Manufacturer


Consult instructions for use


Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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