8-OHdG (8-Hydroxydeoxyguanosine) Competitive ELISA Kit

NB-22-46298-1

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Competitive ELISA Kit

Cat# NB-22-46298-1 size: 96 wells

We highly recommended reading this manual thoroughly before using this kit.

Introduction

This kit is a competitive enzyme immunoassay (ELISA) for in-vitro quantitative measurement of 8-OHdG in serum, plasma, tissue homogenates, cell lysates, cell culture supernatant and other biological fluids..

Principle of The Assay

This ELISA kit uses the Competitive-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with 8-OHdG. A reaction is initiated between biotin labelled 8-OhdG and unlabelled 8-OHdG found in the sample/standard, which compete for a fixed number of sites on a biotinylated detection antibody. After incubation, any excess conjugate and unbound sample or standard are washed away, and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well and incubated. The HRP binds to the biotin before a TMB substrate solution is added and triggers enzymatic digestion of the added substrate. The amount of HRP-bound conjugate is inversely proportional to the concentration of 8-OHdG in the sample. The enzyme-substrate reaction is terminated by the addition of stop solution causing colour change in the wells. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of 8-OHdG in tested samples can be calculated by comparing the OD of the samples to the standard curve.

Sensitivity: 0.94 ng/mL

Detection Limit: 1.56~100 ng/mL

Materials Provided

1) Micro-ELISA Coated Plate — 1 plate, 8×12

   Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20°C for up to 6 months. Reseal along entire edge of zip-seal.

2) Standard (Lyophilized) — x 2

   Aliquot and store at ≤ -20 °C for up to 6 months. * Avoid repeated freeze-thaw cycles.

3) Concentrated Biotinylated Detection Antibody (100×) — 1 ×120 µL

   May be stored for up to 6 months at -20°C. Protect from light.

4) Streptavidin-HRP Concentrated (100×) — 1 ×120 µL

   May be stored for up to 6 months at -20°C. Protect from light.

5) Standard/Sample Diluent — 20 mL

   May be stored for up to 6 months at 2-8°C.

6) Biotinylated Detection Antibody Diluent 14 mL

May be stored for up to 6 months at 2-8°C.

7) Streptavidin-HRP Diluent 14 mL

May be stored for up to 6 months at 2-8°C.

8) Wash Buffer(30x) 30 mL

May be stored for up to 6 months at 2-8°C.

9) TMB Substrate 10 mL

May be stored for up to 6 months at 2-8°C. Protect Substrate from light.

10) Stop Solution 10ml

11) Plate Sealers 2 Strips

Sample Collection and Storage

1. Cell Culture Supernatant

Centrifuge 1000xg for 10 min and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles. If cell culture supernatant samples require larger dilutions, perform an intermediate dilution with culture media and the final dilution with the Standard/Sample Diluent.

2. Serum

Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000xg. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

3. Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately, or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

4. Cell Lysates

Cells need to be lysed before assaying according to the following directions. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1000×g for 5 minutes (suspension cells can be collected by centrifugation directly). Wash cells 3 times in cold PBS. Resuspend cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clarified. Centrifuge at 1500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.

5. Tissue homogenates

The preparation of tissue homogenates will vary depending upon tissue type. Tissues should be rinsed thoroughly in ice-cold PBS to remove excess blood and weighed before homogenization. Mince the tissues to small pieces and homogenise them in fresh lysis buffer (different lysis buffer needs to be chosen

based on subcellular location of the target protein) (E.g., 1mL lysis buffer in 200mg tissue sample) with a glass homogenizer on ice. The resulting suspension should be sonicated with an ultrasonic cell disrupter until the solution is clarified. Centrifuge the homogenates for 5 minutes at 10000×g and collect the supernatant. Assay immediately or aliquot and store at ≤-20°C.

6. Other biological fluids

Centrifuge samples for 20 minutes at 1000×g. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles. **Avoid haemolytic and hyperlipidaemia samples for serum and plasma.**

Dilution: Dilute samples at the appropriate multiple (recommend carrying out a pre-test to determine the dilution factor).

Note

Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months), avoiding freeze-thaw cycles. We recommend predicting the concentration before assaying. If the sample concentration is not within the range of the standard curve users should determine the optimal sample dilutions for their particular experiments. If the sample type is not included in this manual, a preliminary experiment is advised to verify the validity. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation to the results. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

Precautions

1. This kit is for RESEARCH USE ONLY.
2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
3. Variations in sample collection, processing, and storage may cause sample value differences.
4. Reagents may be harmful. If contact made with skin, rinse with an excess amount of tap water.
5. Stop Solution **contains strong acid**. Wear eye, hand, and face protection.
6. For long term storage kit standards should be kept refrigerated, other components should be frozen.
7. Please perform centrifugation to collect liquid before use.
8. Do not mix or substitute reagents with those from other lots or other sources.
9. Adequate mixing is very important for a good result. Use a mini-vortex at the lowest frequency.
10. Mix each sample and all components in the kits adequately and use a clean plastic container to prepare diluent.
11. Samples and standards should be assayed in duplicate, and the sequence of the regents should be added consistently.
12. Reuse of the dissolved standard is not recommended.
13. The kit should not be used beyond the expiration date on the kit label.
14. The kit should be kept away from light when it is stored or incubated.
15. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma 16. and other biological fluids in accordance with appropriate regulations.
16. To avoid cross contamination, please use disposable pipette tips.

17. Please prepare all kit components according to the specification. If the kits will be used several times, keep unused strips sealed and preserve with desiccants. Use within 2 months.

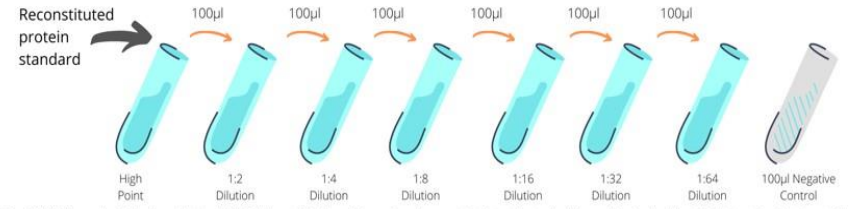
Experiment Materials

The following materials are required to carry out the aforementioned assay but are not included with this kit.

1. Microplate reader (measuring absorbance at 450 nm, with the correction wavelength set at 570 nm or 630 nm).
2. Pipettes and pipette tips: 0.5-10, 2-20, 20-200, 200-1000 µL.
3. Microplate washer, Squirt bottle.
4. Micro-oscillator.
5. Deionized or double distilled water graduated cylinder.
6. Polypropylene Test tubes for dilution.
7. Incubator.

Reagent Preparation

1. Bring all reagents to room temperature before use. If crystals have formed in the concentrate bring the reagent to room temperature and mix gently until the crystals have completely dissolved. It is recommended to test in duplicates.
2. Standard: Add Standard/Sample Diluent 1.0mL into freeze-dried standard, sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (100ng/mL). Prepare EP tubes containing Standard/Sample Diluent, and carry out a serial dilution according to the picture shown below (recommended concentration for standard curve: 100 ng/mL, 50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.13 ng/mL, 1.56 ng/mL). Any remaining standard solution can be aliquoted and stored at -20°C to -70°C.



Two-fold dilution series: Each microvial starts with 100µl diluent. 100µl is carried over from the reconstituted protein standard, thoroughly mixed and then 100µl is transferred to the next. The process is repeated in each microvial in the sequence. No treatment is carried out on the negative control, which should hold 100µl of diluent.

Dilution Method

1. Concentrated Biotin-Conjugated Antibody (100x): Dilute 1:100 with the BiotinConjugate Antibody Diluent before use, and the diluted solution should be used within 30 min.

No. of strips	Concentrated Biotin-Conjugate antibody (100x)	Biotin-Conjugate antibody diluent
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

2. Streptavidin-HRP Concentrated (100x): Dilute 1:100 with the Streptavidin- HRP Diluent before use, and the diluted solution should be used within 30 min

No. of strips	Concentrated Streptavidin-HRP (100x)	Streptavidin-HRP antibody diluent
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

3. Wash buffer: Dilute 1:30 with double distilled or deionized water before use.

Wash Method

Aspirate each well and wash, repeating the process 2 times for a total of 3 washes. Wash by filling each well with Wash Buffer (350µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

Assay Procedure

1. 1. Remove excess microplate strips from the plate frame and return them to the foil pouch containing the desiccant pack, and reseal.
2. Ensure you have determined which wells will contain the diluted standards (recommended in duplicates), the blank and the samples (also recommended in duplicates).
3. Add 50 µL of each concentration of standard and samples to their allotted wells, and then immediately add 50 µL of Biotinylated Detection Antibody working solution to each well. (It is recommended to add solution as close to the bottom of the well as possible, and without touching the sides, to prevent foaming).
4. Cover with the adhesive strip provided and incubate for 45 minutes at 37°C.
5. Remove all the liquid from each well and wash with 350 µL wash buffer, 3 times. Allow to soak for one minute with each wash.
6. Add 100µL HRP-Conjugate Working Solution to all the wells. Cover with new adhesive strip provided and incubate for 30 min at 37°C.
7. Aspirate the solution from all the wells and wash repeat the wash phase.
8. Add 90 µL Substrate Reagent to each well and incubate for 15-20 minutes at 37°C. Protect from light. Note: The reaction time may be shortened or extended according to the actual colour change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.
9. Add 50 µL Stop Solution to each well. Determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If the wavelength correction is not available, subtract the readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

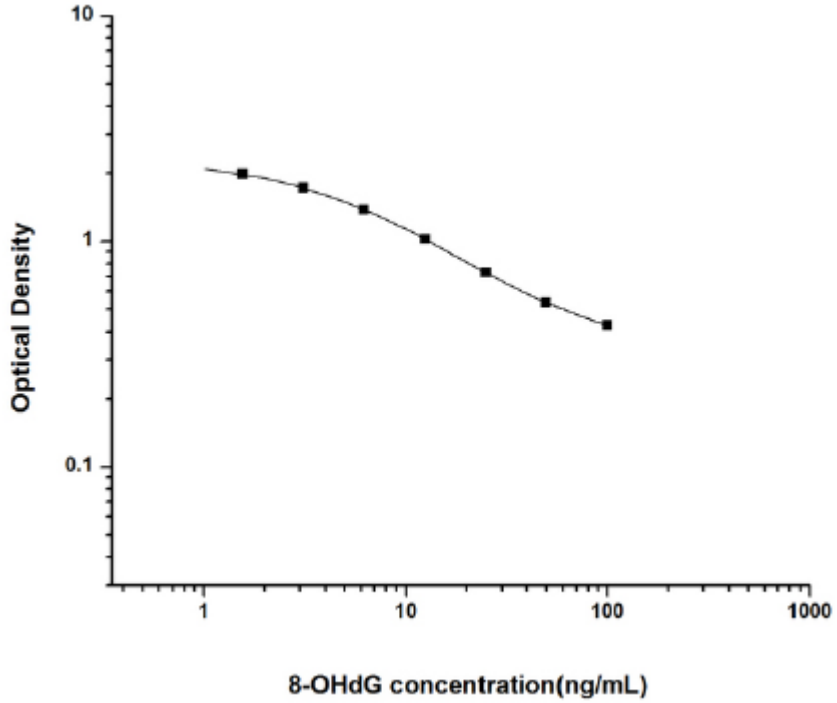
10. Upon completion of the experiment ensure you return unused reagents to their appropriate storage locations.

Calculation of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y- axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the 8-OhdG concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data

The standard curves are provided for demonstration only. A standard curve should be generated for each set of 8-OHdG assayed.



Specificity

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

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid-range and highlevel 8-OHdG were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid-range and highlevel 8-OHdG were tested on 3 different plates, 20 replicates in each plate.

Inter-plate Precision

Inter-assay Precision: 3 samples with low, mid-range and high-level 8-OHdG were tested on 3 different plates, 20 replicates in each plate.

Recovery

The recovery of 8-OHdG spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Matrix	Recovery range (%)	Average (%)
Serum (n=8)	86-100	91
EDTA plasma (n=8)	91-104	98
Cell culture media (n=8)	93-16	98

Troubleshooting

Problem	Causes	Solutions
Poorly developed standard curve	Inaccurate pipetting.	Check pipetting volume consistency and accuracy.
	Improper standard dilution.	Gently mix the standard solution and dissolve the powder thoroughly in solution.
	Wells were not fully aspirated.	Completely aspirate wells in between stages.
Low fluorescence readings	Insufficient incubation time.	Ensure sufficient incubation time.
	Incorrect assay temperature.	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes or inconsistent dilution.	Check pipettes and ensure correctly prepared.
Large CV	Inaccurate pipetting.	Check pipettes and technique.
High background	Concentration of target protein is too high.	Use recommended dilution factor.
	Plate is insufficiently washed.	Review the manual's washing process. If using a plate washer, check that the ports are not obstructed.
	Contaminated wash buffer.	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the kit.	All the reagents should be stored according to the instructions.
	Too long incubation time.	Ensure precise incubation time.