Deoxyribonuclease-2-alpha (DNase II alpha) (Human) ELISA Kit

04/20

(Catalog # E4820-100, 96 assays, Store at 4°C)

I. Introduction:

Deoxyribonuclease-2-alpha hydrolyzes DNA under acidic conditions with a preference for double-stranded DNA. It plays a major role in the degradation of nuclear DNA in cellular apoptosis during development. DNase II alpha is necessary for proper fetal development and for definitive erythropoiesis in fetal liver, where it degrades nuclear DNA expelled from erythroid precursor cells. BioVision's Deoxyribonuclease-2-alpha (DNase II alpha) (Human) ELISA Kit is based on the Sandwich ELISA Principle. A 96-well plate was coated with a capture antibody and the biotin conjugated antibody was used as detection antibody. The standards, test samples, and biotin conjugated detection antibody were added to the wells subsequently and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding the acidic stop solution. The density of color is proportional to the amount of DNase II alpha captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Human DNase II alpha
Detection Range: 78.13-5000 pg/ml
Sensitivity: 46.88 pg/ml
Precision: Intra-Assay: CV<8%, Inter-Assay: CV<10%
This assay has high sensitivity and specificity for detection of DNase II. No significant crossreactivity or interference between DNase II and analogues was observed.

III. Sample Type:

Serum, plasma, tissue homogenates and other biological fluids

IV. Kit Contents:

Components	E4820-100	Part No.	Storage Temp.
Micro ELISA Plate	8 X 12 strips	E4820-100-1	-20°C
Lyophilized Standard (5000 pg)	2 vials	E4820-100-2	-20°C
Sample / Standard dilution buffer	20 ml	E4820-100-3	4°C
Biotin- labeled antibody	120 µl	E4820-100-4	4°C
Antibody dilution buffer	10 ml	E4820-100-5	4°C
HRP-Streptavidin Conjugate (SABC)	120 µl	E4820-100-6	4°C (Avoid light)
SABC dilution buffer	10 ml	E4820-100-7	4°C
TMB substrate	10 ml	E4820-100-8	4°C (Avoid light)
Stop Solution	10 ml	E4820-100-9	4°C
Wash buffer (25X)	30 ml	E4820-100-10	4°C
Plate sealers	5	E4820-100-11	4°C

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

An unopened kit can be stored at 4°C for up to 6 months. If the kit is not used within 1 month, store the items separately according to the above mentioned conditions once the kit is received.

VII. Reagent and Sample Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Biotin- detection antibody working solution**: Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.
2. **HRP-Streptavidin Conjugate (SABC)**: Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.

3. **Wash Buffer**: Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, warm it with 40°C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.
4. **Standard Preparation:**
   - Add 1 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly.
   - Label 7 tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 ml of the Sample Dilution Buffer into each tube. Add 0.3 ml of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly.
   - Transfer 0.3 ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control. (Note: Please use Standard Solutions within 2 hours of preparation).
5. **Sample Preparation:**

Note: Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C (≤1 month) or -80°C (≤2 months). Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

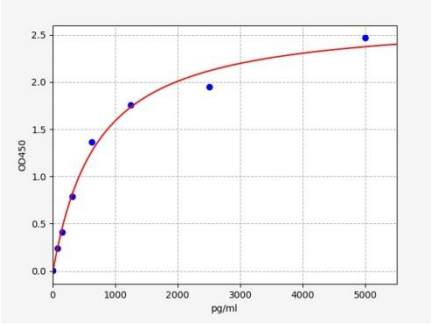
- **Serum**: Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000xg, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and endotoxin free.
- **Plasma:** Collect plasma using EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000xg at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Tissue homogenates**: As hemolysis blood has relation to assay result, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue. Normal, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5minutes at 5000×g to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.
- **Cell culture supernatant**: Centrifuge supernatant for 20 minutes at 1000×g at 2 - 8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.
- **Cell Culture Lysate:** Commercial RIPA kits are recommended to follow the instructions provided. Generally, 0.5 ml RIPA lysis buffer would be appropriate to $2x10^6$ cells, DNA must to be removed. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg.
- **Other biological fluids:** Centrifuge samples for 20 min at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.

Note: End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve should be run for each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Wash plate 2 times with **1X Wash buffer** before adding standard, sample and control wells.
3. Add 100 µl of each **standards** or **samples** into appropriate wells. Cover well and incubate for 1.5 hours at 37ºC.
4. Remove the cover and discard the plate content. Wash the plate 2 times with **1x wash buffer** without letting the wells completely dry.
5. Add 0.1 ml of **Biotin-detection antibody** work solution into the above wells. Seal the plate and incubate at 37ºC for 60 min.
6. Discard the solution and wash 3 times with **1X Wash buffer**. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or autowasher. Let it soak for 1-2 minutes, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
7. Add 0.1 ml of **SABC working solution** into each well, cover the plate and incubate at 37ºC for 30 min.
8. Discard the solution and wash 5 times with **1X Wash buffer** as step 6.
9. Add 90 µl of **TMB substrate** into each well, cover the plate and incubate at 37 ºC in dark within 10-20 min. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. The reaction can be terminated when apparent gradient appeared in standard wells).
10. Add 50 µl of **Stop Solution** to each well. Read result at 450 nm within 20 minutes.



IX. Calculation

For calculation, **(the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well).** The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. If the samples

measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

STD.(pg/ml)	OD-1	OD-2	Average	Corrected
0	0.006	0.006	0.006	0.000
78.125	0.235	0.241	0.238	0.232
156.25	0.406	0.418	0.412	0.406
312.5	0.775	0.797	0.786	0.78
625	1.346	1.384	1.365	1.359
1250	1.733	1.783	1.758	1.752
2500	1.923	1.979	1.951	1.945
5000	2.436	2.506	2.471	2.465

Figure: Typical Standard Curve and OD values: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. Recovery:

Matrices listed below were spiked with certain level of DNase II and the recovery rates were calculated by comparing the measured value to the expected amount of DNase II in samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	89-103	95
EDTA Plasma(n=5)	85-97	92
Heparin Plasma(n=5)	86-98	93

XI. Linearity:

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of DNase II and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
Serum(n=5)	86-97%	89-102%	86-94%
EDTA Plasma(n=5)	91-98%	83-91%	85-92%
Heparin Plasma(n=5)	87-100%	82-99%	80-98%

XII. RELATED PRODUCTS:

- Deoxyribonuclease-1 (DNase1) (Human) ELISA Kit (E4500)
- DNase I Antibody (3214)
- DNAse I Activity Assay Kit (Fluorometric) (K429)
- RNase-Free DNase I Set (M1229)