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11-Dehydrothromboxane B_2 ELISA Kit

06/20

(Catalog # E4900-100; 100 assays; Store at -20°C)

I. Introduction:

11-Dehydrothromboxane B_2 (11dhTxB_2 or 11-dehydro-TxB_2) is a stable metabolite of Thromboxane A_2 (TxA_2). TxA_2 is a prothrombotic agent capable of promoting the activation and subsequent aggregation of platelets nearby. It is derived from Arachidonic Acid in the COX pathway and is involved in maintaining hemostasis & preventing cardiovascular diseases. TxA_2 has a very short half-life and is quickly hydrolyzed to thromboxane B_2 (TxB_2), which further metabolizes to 11dhTxB_2. 11dhTxB_2 has a stable half-life and is excreted in the urine. It is unaffected by the *ex vivo* platelet activation and other pre-analytical variables. Urinary 11dhTxB_2 can directly reflect the platelet production of TxA_2 and represents a good and reliable biomarker for platelet activity. Additionally, Aspirin can irreversibly inhibit the platelet COX-1 activity thereby resulting in the inhibition of TxA_2 production and thromboxane-mediated platelet activation. Thus, urine levels of 11dhTxB_2 reflect COX-1 inhibition and can be used to monitor responses to Aspirin therapy. **BioVision's 11-Dehydrothromboxane B_2 ELISA Kit** can quickly and reliably determine a broad range of 11dhTxB_2 concentrations in human urine samples. It is a competitive ELISA, based on the competition between the free 11dhTxB_2 and 11dhTxB_2-Acethylcholinesterase (AChE) conjugate for a constant amount of 11dhTxB_2 monoclonal antibody binding sites. Thus, in the presence of an AChE substrate, the absorbance of the released product is inversely proportional to the amount of 11dhTxB_2 in samples. The kit can detect as low as 15.6 pg/ml of 11dhTxB_2 in 60 minutes.

II. Application:

- The ELISA kit is used for *in vitro* quantitative determination of 11dhTxB_2.

III. Specificity:

- Human

IV. Sample Type:

- Human Urine

V. Kit Contents:

Components	E4900-100	Cap Code	Part Number
Pre-coated 96 well strip plate	8 x 12 strips	--	E4900-100-1
11dhTxB_2 Standard	300 µl	Black	E4900-100-2
11dhTxB_2-AChE	1 vial	Orange	E4900-100-3
11dhTxB_2 Monoclonal Antibody	50 µl	Green	E4900-100-4
11dhTxB_2 Incubation Buffer (10X)	25 ml	WM	E4900-100-5
AChE Substrate	1 vial	Purple	E4900-100-6
AChE Probe	1 vial	Red	E4900-100-7
11dhTxB_2 Developer	25 ml	NM	E4900-100-8
Wash Buffer (10X)	30 ml	NM	E4900-100-9
Plate Sealers	4	--	E4900-100-10

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 412 nm
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended
- Deionized water
- Eppendorf tubes
- 15 ml conical tubes
- Absorbent paper

VII. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Upon opening, use within two months.

- **11dhTxB_2 Standard (200 ng/ml):** Store at -20ºC.
- **11dhTxB_2 Monoclonal Antibody:** Store at -20ºC. Keep on ice when in use.
- **11dhTxB_2 Incubation Buffer (10X) & 11dhTxB_2 Developer:** Store at 4ºC. Prepare **1X11dhTxB_2 Incubation Buffer** by diluting 11dhTxB_2 Incubation Buffer (10X) with dH_2O. The 1xBuffer can be store at 4ºC for one month. Bring Buffer to room temperature (RT) before use.
- **11dhTxB_2-AChE:** Reconstitute the vial in 520 µl of **1X11dhTxB_2 Incubation Buffer**. Divide into aliquots and store at -20ºC.
- **AChE Substrate:** Reconstitute the vial in 100 µl of **11dhTxB_2 Developer**. Store at -20ºC**,** protected from light.
- **AChE Probe:** Reconstitute the vial in 625 µl of **11dhTxB_2 Developer**. Store at -20ºC. Use within two months.
- **Wash Buffer (10X):** Bring the bottle to RT. If crystals are present, warm up to RT and mix gently until the crystals are completely dissolved. Prepare 1X Wash Buffer for the ELISA by diluting Wash Buffer (10X) with dH_2O. The 1X solution can be stored at 4ºC for one month.

VIII. 11dhTxB_2 Standard Preparation:

1. Prepare 40 µl of 20 ng/ml 11dhTxB_2 Standard by mixing 4 µl of 200 ng/ml 11dhTxB_2 Stock Standard with 36 µl dH_2O.
2. Prepare 10 ml 1x11dhTxB_2 Incubation Buffer by diluting 1 ml of 11dhTxB_2 (10X) with 9 ml dH_2O.
3. Prepare 300 µl of 2000 pg/ml 11dhTxB_2 Standard by mixing 30 µl of 20 ng/ml Standard with 270 µl of 1X11dhTxB_2 Incubation Buffer.
4. Perform 2-fold serial dilutions of the Standards (as shown below) to make the Standard Curve within the range of the assay. Suggested Standard points are 2000, 1000, 500, 250, 125, 62.5, 31.3 and 15.6 pg/ml. Incubate the Standards at RT for a minimum of one hour before adding to 96-well strip plate.

Standard	S1	S2	S3	S4	S5	S6	S7	S8
11dhTxB$_2$ Incubation Buffer (1X)	270 µl	150 µl	150 µl	150 µl	150 µl	150 µl	150 µl	150 µl
11dhTxB$_2$ Standard (20 ng/ml)	30 µl							
		150 µl S1	150 µl S2	150 µl S3	150 µl S4	150 µl S5	150 µl S6	150 µl S7
	Mix well							
Final Standard Conc. (pg/ml)	**2000**	**1000**	**500**	**250**	**125**	**62.5**	**31.3**	**15.6**

IX. Sample Preparation:

Human Urine:

1. Centrifuge the Sample(s) at 2000 - 3000 rpm at 4ºC for 20 min to remove any precipitated proteins. Collect the supernatant carefully.
2. Dilute the supernatant a minimum of 1:2 ratio with 1X11dhTxB$_2$ Incubation Buffer. Incubate the diluted sample(s) at RT for a minimum of one hour before adding to the 96-well strip plate.

Note: E4900-100 is only for human samples. Samples of mouse or rat origin may contain antibodies which can interfere with the assay.

X. 11dhTxB$_2$ ELISA Assay Protocol:

Notes:

a. A Standard Curve must be run with each assay.
b. Bring all Buffer(s) to RT 30 minutes prior to the assay.
c. Prepare 100 ml **1X Wash Buffer** by diluting 10 ml of Wash Buffer (10X) with 90 ml dH$_2$O.
d. Prepare 10 ml **1X11dhTxB$_2$ Incubation Buffer** by diluting 1 ml of 11dhTxB$_2$ Incubation Buffer (10X) with 9 ml dH$_2$O.
e. Prepare 1:10 dilution of **11dhTxB$_2$-AChE** (i.e. dilute 50 µl of reconstituted 11dhTxB$_2$-AChE with 450 µl 1X11dhTxB$_2$ Incubation Buffer).
f. Prepare 1:100 dilution of **11dhTxB$_2$ Monoclonal Antibody** (i.e. dilute 5 µl of 11dhTxB$_2$ Monoclonal Antibody Stock Solution with 495 µl 1X11dhTxB$_2$ Incubation Buffer).

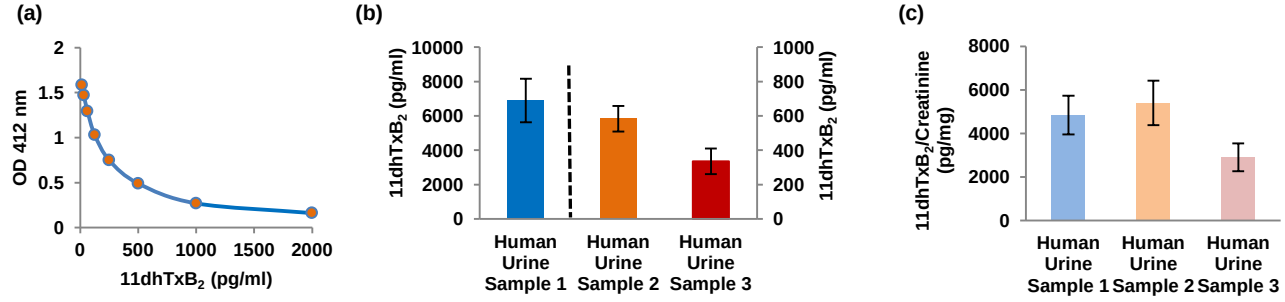
1. Prepare all the Reagents, Standards and Sample(s) as instructed.
2. Add 50 µl of **Standards** or **Sample(s)** into appropriate wells.
3. Add 100 µl **1X11dhTxB$_2$ Incubation Buffer** into the **Background Control** well(s).
4. Add 50 µl of **Diluted 11dhTxB$_2$-AChE** to each well including Standards, Sample(s) and Background Control. **Shake the plate gently at RT for 5-10 min.**
5. Add 50 µl of **Diluted 11dhTxB$_2$ Monoclonal Antibody** into Standards and Sample(s) wells. *The total volume in each well is 150 µl*.
Note: Do not add Diluted 11dhTxB$_2$ Monoclonal Antibody to Background Control well.
6. Cover the plate with a plate sealer. Incubate the plate by shaking gently at RT for 2 hours, avoid light.
7. Aspirate all the reagents and wash each well **5** times: Wash by filling each well with 250 µl of **1X Wash Buffer** and incubating for 3-5 min. Remove the 1X Wash buffer completely before the next wash. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
Note: Complete removal of wash buffer is essential for accurate results.
8. Prepare 12-fold dilution of the **AChE Substrate** by mixing 10 µl of AChE Substrate with 110 µl of **11dhTxB$_2$ Developer**. Mix enough reagents for the number of assays to be performed. For each well, prepare 200 µl Reaction Mix:

	Reaction Mix
Diluted AChE Substrate	10 µl
AChE Probe	5 µl
11dhTxB$_2$ Developer	185 µl

Mix and add 200 µl Reaction Mix into all wells. Tap or shake the plate to ensure complete mixing. ***The total final reaction volume of each well will be 200 µl***. Incubate the plate at RT in dark for 60 minutes.
9. Measure the OD at 412 nm at RT in end point mode.

XI. Calculation:

Subtract the Background Control reading from all the Standards and Sample(s) Readings. Plot the 11dhTxB$_2$ Standard Curve as the relative OD$_{412\ nm}$ of each Standard solution (Y) vs. the respective concentration of the Standards (X). The 11dhTxB$_2$ concentration of the samples can be interpolated from the 11dhTxB$_2$ Standard Curve. **Note:** If the Sample(s) are diluted, multiply the dilution factor to the concentrations from interpolation. The concentration of 11dhTxB$_2$ in Sample(s) is calculated from the 11dhTxB$_2$ Standard Curve and can be reported as pg of 11dhTxB$_2$ per mg Creatinine to normalize the results combining with Creatinine Assay.



Figures. **a.** 11dhTxB$_2$ Standard Curve. *This Standard Curve is for demonstration only. A Standard Curve must be run with each assay*. **b.** 11dhTxB$_2$ amounts (pg/ml) in Human Urine samples (1:10; 1:2 and 1:5 dilutions respectively). **c**. Results are normalized as pg of 11dhTxB$_2$ per mg creatinine by using Creatinine Colorimetric/Fluorometric Assay Kit (BioVision Cat# K625).

XII. Related Products:

Creatinine Colorimetric/Fluorometric Assay Kit (K625)
Creatinine (Human) ELISA Kit (E4368)
Creatinine (Mouse) ELISA Kit (E4369)
Creatinine (Rat) ELISA Kit (E4370)

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