# 11-dehydro-TXB2 ELISA Kit

Catalog Number KA0314

96 assays

Version: 02

Intended for research use only

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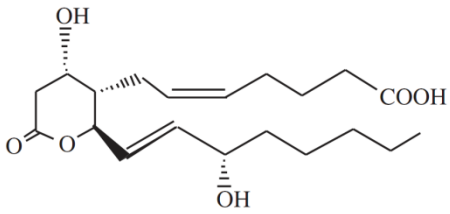
# Table of Contents

## Introduction

### Background

11-dehydro-Thromboxane B2 (11-dehydro-TXB2) is a major metabolite of Thromboxane B2 (TXB2) found in urine and plasma[3-12]. It is produced by the dehydrogenation of the alcohol group on the C11 position of TXB2 by the enzyme 11-OH-dehydrogenase[3]. The detection of 11-dehydro-TXB2 is often used to measure thromboxane production in vivo[3-4, 5,8]. Monitoring levels of this metabolite is a helpful tool in the study of several diseases, such as liver cirrhosis[5], cystic fibrosis6, mastocytosis[7], systemic lupus erythematosus[8], thrombosis diseases[9], and other diseases involving platelet activation[3, 10]. 11-dehydro-TXB2 has also been used in diabetes[11] and asthma studies[12].

11-dehydro-Thromboxane B2




### Principle of the Assay

The 11-dehydro-Thromboxane B2 kit is a competitive immunoassay for the quantitative determination of 11-dehydro-Thromboxane B2 in tissue culture media and urine. Please read the complete kit insert before performing this assay. The kit for the quantitative measurement of 11-dehydro-Thromboxane B2 uses a polyclonal antibody to 11-dehydro-TXB2 to bind, in a competitive manner, the 11-dehydro-TXB2 in the sample or an alkaline phosphatase molecule which has 11-dehydro-TXB2 covalently attached to it. After a simultaneous incubation at room temperature the excess reagents are washed away and substrate is added. After a short incubation time the enzyme reaction is stopped and the yellow color generated is read on a microplate reader at 405nm. The intensity of the bound yellow color is inversely proportional to the concentration of 11-dehydro-TXB2 in either standards or samples. The measured optical density is used to calculate the concentration of 11-dehydro-TXB2. For further explanation of the principles and practice ofimmunoassays please see the excellent books by Chard[1] or Tijssen[2].

## General Information

### Materials Supplied

List of component

| Component | Amount |
|---|---|
| Goat anti-Rabbit IgG Microtiter Plate: A plate using break-apart strips coated with goat antibody specific to rabbit IgG. | 96 (8 x 12) Wells |
| 11-dehydro-TXB2 EIA Conjugate: A blue solution of alkaline phosphatase conjugated with 11-dehydro-TXB2. | 6 mL |
| 11-dehydro-TXB2 EIA Antibody: A yellow solution of a rabbit polyclonal antibody to 11-dehydro-TXB2 | 6 mL |
| Assay Buffer: Tris buffered saline, containing proteins and sodium azide as preservative. | 30 mL |
| Wash Buffer Concentrate: Tris buffered saline containing detergents. | 30 mL |
| 11-dehydro-Thromboxane B2 Standard: A solution of 100,000 pg/mL 11-dehydro-TXB2 | 0.5 mL |
| pNpp Substrate: A solution of p-nitrophenyl phosphate in buffer. Ready to use. | 20 mL |
| Stop Solution: A solution of trisodium phosphate in water. Keep tightly capped. Caution: Caustic. | 5 mL |
| Plate Sealer | 1 each |

### Storage Instruction

All components of this kit, except the Conjugate and the Standard, are stable at 4℃ until the kit's expiration date. The Conjugate and Standard must be stored frozen at -20℃.

### Materials Required but Not Supplied

- ✓ Deionized or distilled water.
- ✓ Precision pipets for volumes between 5 µL and 1,000 µL.
- ✓ Repeater pipets for dispensing 50 µL and 200 µL.
- ✓ Disposable beaker for diluting buffer concentrates.
- ✓ Graduated cylinders.
- ✓ A microplate shaker.
- ✓ Adsorbent paper for blotting.
- ✓ Microplate reader capable of reading at 405 nm, preferably with correction at between 570 and 590 nm.

## Precautions for Use

- Precautions

  FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

✓ Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up.

✓ Stop solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.

✓ The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg2+ and Zn2+ ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.

✓ We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.

✓ The 11-dehydro-Thromboxane B2 Standard provided is supplied in ethanolic buffer at a pH optimized to maintain 11-dehydro-TXB2 integrity. Care should be taken handling this material because of the known and unknown effects of eicosanoids.

*Procedure Note:*

✓ *Do not mix components from different kit lots or use reagents beyond the kit expiration date.*

✓ *Allow all reagents to warm to room temperature for at least 30 minutes before opening.*

✓ *Standards can be made up in either glass or plastic tubes.*

✓ *Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.*

✓ *Pipet standards and samples to the bottom of the wells.*

✓ *Add the reagents to the side of the well to avoid contamination.*

✓ *This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4 ℃ in the sealed bag provided. The wells should be used in the frame provided.*

✓ *Care must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.*

✓ *Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.*

## Assay Protocol

### Reagent Preparation

- 11-dehydro-TXB2 Standard

  Allow the 100,000 pg/mL 11-dehydro-TXB2 standard solution to warm to room temperature. Label six 12 x 75 mm glass tubes #1 through #6. Pipet 1,000 µL of standard diluent (Assay Buffer or Tissue Culture Media) into tube #1. Pipet 750 µL of standard diluent into tubes #2 through #6. Remove 100 µL of diluent from tube #1. Add 100 µL of the 100,000 pg/mL standard to tube #1. Vortex thoroughly. Add 250 µL of tube #1 to tube #2 and vortex thoroughly. Add 250 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through#6.

  The concentration of 11-dehydro-TXB2 in tubes #1 through #6 will be 10,000, 2,500, 625,156.3, 39.1 and 9.8 pg/mL respectively.

  Diluted Standards should be used within 60 minutes of preparation.

- Wash Buffer

  Prepare the Wash Buffer by diluting 5 mL of the supplied concentrate with 95 mL of deion-ized water. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

### Sample Preparation

The 11-dehydro-TXB2 enzyme immunoassay is compatible with 11-dehydro-TXB2 samples in tissue culture media and human urine. Samples diluted sufficiently into Assay Buffer can be read directly from the standard curve. Please refer to the Sample Recovery recommendations for details of suggested dilutions. However, the end user must verify that the recommended dilutions are appropriate for their samples. Samples containing rabbit IgG may interfere with the assay.

Samples in the majority of tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Assay Buffer. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of 11-dehydro-TXB2 in the appropriate matrix. For tissue and urine samples, prostaglandin synthetase inhibitors, such as indomethacin or meclofenamic acid at concentrations up to 10 µg/mL, should be added to either the tissue homogenate or urine samples. Urine samples may be used in the assay without dilution. Some samples may require extraction for accurate measurement. A suitable extraction procedure is outlined below:

- Materials Needed

✓ 11-dehydro-TXB2 Standard to allow extraction efficiency to be accurately determined.

✓ 2M hydrochloric acid, deionized water, ethanol, hexane and ethyl acetate.

✓ 200 mg C18 Reverse Phase Extraction Columns.

- Procedure

1. Acidify the urine or tissue homogenate by addition of 2M HCl to pH of 3.5. Allow to sit at 4 ℃ for 15 minutes. Centrifuge samples in a microcentrifuge for 2 minutes to remove any precipitate.
2. Prepare the C18 reverse phase column by washing with 10 mL of ethanol followed by 10 mL of deionized water.
3. Apply the sample under a slight positive pressure to obtain a flow rate of about 0.5 mL/minute. Wash the column with 10 mL of water, followed by 10 mL of 15% ethanol, and finally 10 mL hexane. Elute the sample from the column by addition of 10 mL ethyl acetate.
4. If analysis is to be carried out immediately, evaporate samples under a stream of nitrogen. Add at least 250 µL of Assay Buffer to the dried samples. Vortex well then allow to sit for 5 minutes at room temperature. Repeat twice more. If analysis is to be delayed, store samples as the eluted ethyl acetate solutions at -80 ℃ until the immunoassay is to be run. Evaporate the organic solvent under a stream of nitrogen prior to running assay and reconstitute as above.

Please refer to references 13 and 14 for details of extraction protocols.

## Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4 ℃.
2. Pipet 100 µL of Standard Diluent (Assay Buffer or Tissue Culture Media) into the NSB and the Bo (0 pg/mL Standard) wells.
3. Pipet 100 µL of Standards #1 through #6 into the appropriate wells.
4. Pipet 100 µL of the Samples into the appropriate wells.
5. Pipet 50 µL of Assay Buffer into the NSB wells.
6. Pipet 50 µL of blue Conjugate into each well, except the Total Activity (TA) and Blank wells.
7. Pipet 50 µL of yellow Antibody into each well, except the Blank, TA and NSB wells.

*NOTE: Every well used should be Green in color except the NSB wells which should be Blue. The Blank and TA wells are empty at this point and have no color.*

8. Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided, if so desired.
9. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 2 more times for a total of 3 Washes.
10. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
11. Add 5 µL of the blue Conjugate to the TA wells.
12. Add 200 µL of the pNpp Substrate solution to every well. Incubate at room temperature for 1 hour without shaking.
13. Add 50 µL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.
14. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

## Data Analysis

### Calculation of Results

Several options are available for the calculation of the concentration of 11-dehydro-TXB2 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If this sort of data reduction software is not readily available, the concentration of 11-dehydro-TXB2 can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:
   Average Net OD = Average Bound OD - Average NSB OD
2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:
   Percent Bound = Net OD/ Net Bo OD x 100
3. Using Logit-Log paper plot Percent Bound versus Concentration of 11-dehydro-TXB2 for the standards. Approximate a straight line through the points. The concentration of 11-dehydro-TXB2 in the unknowns can be determined by interpolation.

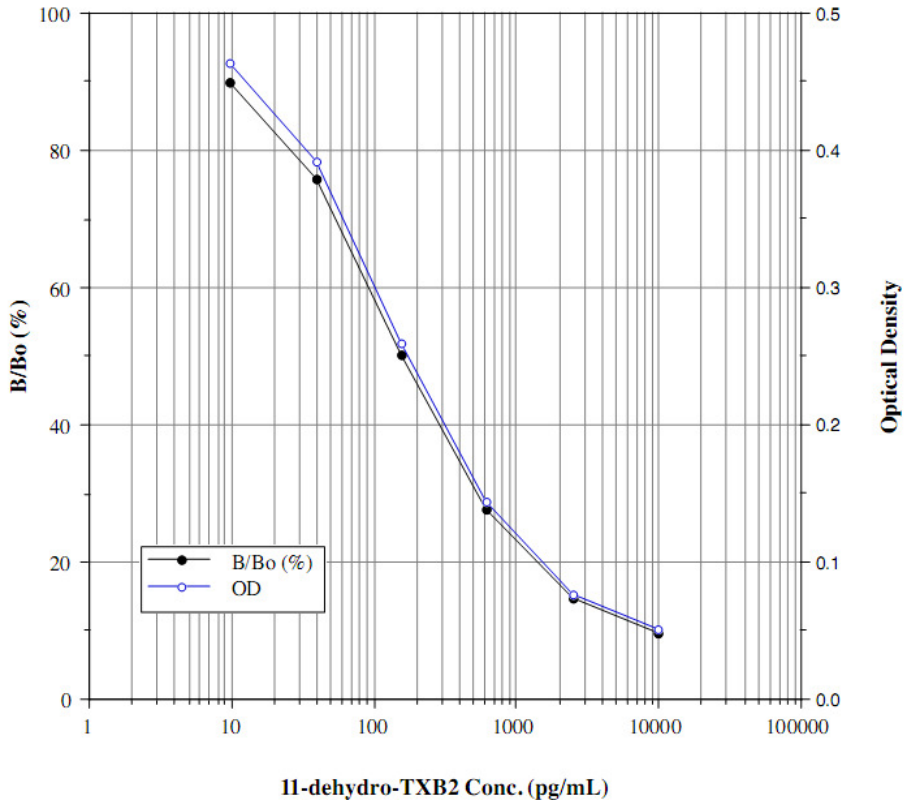
- Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

| | Mean | Average | Percent | 11-dehydro-TXB2 |
|---|---|---|---|---|
| Sample | OD (-Blank) | Net OD | Bound | (pg/mL) |
| Blank OD | (0.093) | | | |
| TA | 0.125 | | | |
| NSB | -0.003 | 0.000 | | |
| Bo | 0.509 | 0.512 | 100% | 0 |
| S1 | 0.047 | 0.050 | 9.8% | 10,000 |
| S2 | 0.074 | 0.077 | 15.0% | 2,500 |
| S3 | 0.136 | 0.139 | 27.1% | 625 |
| S4 | 0.256 | 0.259 | 50.6% | 156.3 |
| S5 | 0.384 | 0.387 | 75.6% | 39.1 |
| S6 | 0.456 | 0.459 | 89.6% | 9.8 |
| Unknown 1 | 0.110 | 0.113 | 22.1% | 966 |
| Unknown 2 | 0.212 | 0.215 | 42.0% | 246 |

- Typical Standard Curve

A typical standard curve is shown below. This curve must not be used to calculate 11-dehydro-TXB2 concentrations; each user must run a standard curve for each assay.




- Typical Quality Control Parameters

| | | |
|---|---|---|
| Total Activity Added | = | 0.125 x 10 = 1.25 |
| %NSB | = | 0.0% |
| %Bo/TA | = | 41.0% |
| Quality of Fit | = | 1.0000 (Calculated from 4 parameter logistic curve fit) |
| 20% Intercept | = | 1,212 pg/mL |
| 50% Intercept | = | 162 pg/mL |
| 80% Intercept | = | 28 pg/mL |

**Performance Characteristics**

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[15].

- Sensitivity

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run as Bo, and comparing to the average optical density for sixteen (16) wells run with Standard #6. The detection limit was determined as the concentration of 11-dehydro-TXB2 measured at two (2) standard deviations from the

zero along the standard curve.

| | | | | |
|---|---|---|---|---|
| Average Optical Density for the Bo | = | 0.507 ± 0.011 (2.26%) | | |
| Average Optical Density for Standard #6 | = | 0.457 ± 0.009 (1.96%) | | |
| Delta Optical Density (0-9.8 pg/mL) | = | 0.050 | | |
| 2 SD's of the Zero Standard | = | 2 x 0.011 | = | 0.022 |
| Sensitivity | = | 0.022/0.050 x 9.8 pg/mL | = | 4.31 pg/mL |

- Linearity

A sample containing 6,613 pg/mL 11-dehydro-TXB2 was diluted serially 1:2 six times with kit Assay Buffer and measured in the assay. The data was plotted graphically as actual 11-dehydro-TXB2 Concentration versus measured 11-dehydro-TXB2 Concentration.

The line obtained had a slope of 0.943 with a correlation coefficient of 0.999.

- Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of 11-dehydro-TXB2 and running these samples multiple times (n=16) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of 11-dehydro-TXB2 in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of 11-dehydro-TXB2 determined in these assays as calculated by a 4 parameter logistic curve fitting program.

| | 11-dehydro-TXB2 (pg/mL) | Intra-assay %CV | Inter-assay %CV |
|---|---|---|---|
| Low | 67 | 13.2 | |
| Medium | 165 | 15.3 | |
| High | 318 | 8.1 | |
| Low | 72 | | 17.5 |
| Medium | 216 | | 12.6 |
| High | 1,250 | | 16.3 |

- Cross Reactivities

The cross reactivities for a number of related eicosanoid compounds was determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer at concentrations from 100,000 to 1.0 pg/mL. These samples were then measured in the 11-dehydro-TXB2 assay, and the measured 11-dehydro-TXB2 concentration at 50% B/Bo calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

| Compound | Cross Reactivity |
|---|---|
| 11-dehydro TXB2 | 100% |
| PGE2 | 1.85% |
| TXB2 | 0.4% |
| PGF2α | 0.2% |
| 6-keto-PGF1α | 0.17% |
| PGD2 | 0.1% |
| 2,3-dinor TXB2 | 0.1% |
| 8-iso-PGF2α | 0.07% |
| 11-b-PGF2α | 0.05% |

- Sample Recoveries

Please refer to Sample Handling and Standard Preparation.

11-dehydro-TXB2 concentrations were measured in tissue culture media and human urine. For samples in tissue culture media, ensure that the standards have been diluted into the same media. For all of the samples, 11-dehydro-TXB2 was spiked into the samples which were diluted with the kit Assay Buffer or media. The following results were obtained:

| Sample | % Recovery* | Recommended Dilution* |
|---|---|---|
| Tissue Culture Media | 104.8 | None |
| Human Urine | 112.2 | 1:32 |

* See Sample Handling instructions for details.

## Resources

### References


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**Plate Layout**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Blank | Std 1 | Std 5 | | | | | | | | | |
| B | Blank | Std 1 | Std 5 | | | | | | | | | |
| C | TA | Std 2 | Std 6 | | | | | | | | | |
| D | TA | Std 2 | Std 6 | | | | | | | | | |
| E | NSB | Std 3 | | | | | | | | | | |
| F | NSB | Std 3 | | | | | | | | | | |
| G | Bo | Std 4 | | | | | | | | | | |
| H | Bo | Std 4 | | | | | | | | | | |