



Product Manual

TXB₂ ELISA kit

Catalog #: ADI-900-002

96 Well Kit



Product Manual

USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON-INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

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



Please read entire booklet before proceeding with the assay.



Carefully note the handling and storage conditions of each kit component.



Please contact Enzo Life Sciences Technical Support if necessary.

TABLE OF CONTENTS

Description	2
Introduction	2
Precautions	3
Materials Supplied.....	4
Storage	5
Other Materials Needed	5
Sample Handling.....	6
Procedure	7
Procedural Notes	8
Reagent Preparation	9
Assay Procedure.....	9
Calculation of Results	11
Typical Results.....	11
Performance Characteristics	13
Sample Dilution Recommendations	15
References.....	16
Contact Information.....	18

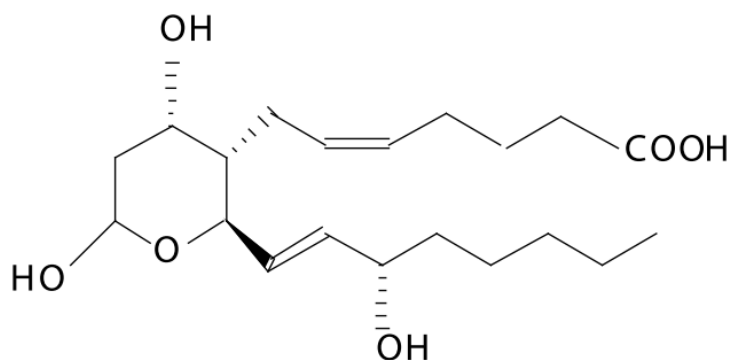
DESCRIPTION

The Thromboxane B₂ enzyme-linked immunosorbent assay (ELISA) kit is a competitive immunoassay for the quantitative determination of Thromboxane B₂ in biological fluids. Please read the complete kit insert before performing this assay. The kit for the quantitative measurement of Thromboxane B₂ uses a polyclonal antibody to TXB₂ to bind, in a competitive manner, the TXB₂ in the sample or an alkaline phosphatase molecule which has 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 read on a microplate reader at 405nm. The intensity of the bound yellow color is inversely proportional to the concentration of TXB₂ in either standards or samples. The measured optical density for the samples is used to calculate the concentration of TXB₂ in the sample. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard¹ or Tijssen².

INTRODUCTION

Thromboxane A₂ (TXA₂) is involved in platelet aggregation, vasoconstriction and reproductive functions³⁻⁵. However TXA₂ has a half-life of only 37 seconds under physiological conditions⁵, and the production of TXA₂ in vivo is typically monitored by measurement of TXB₂ and 2,3-dinor TXB₂. Thromboxane B₂ (TXB₂) is produced by the non-enzymatic hydration of TXA₂, and has been shown to be stable^{6,7}. A number of pharmaceuticals alter and/or inhibit the synthesis of TXA₂⁸⁻¹⁰ and methods to determine TXA₂ in urine and blood typically involve gas chromatography/mass spectrometry¹¹, radioimmunoassay, or enzyme-linked immunosorbent assay.

Thromboxane B₂



SAFETY WARNINGS & 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.
- The Thromboxane B2 Standard provided, Catalog No. 80-0007, is supplied in ethanolic buffer at a pH optimized to maintain TXB2 integrity. Care should be taken handling this material because of the known and unknown effects of eicosanoids.

MATERIALS SUPPLIED

1. **Goat anti-Rabbit IgG Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-0060**
A plate using break-apart strips coated with goat antibody specific to the Fc portion of rabbit IgG.
2. **TXB₂ ELISA Conjugate, 5 mL, Catalog No. 80-0005**
A blue solution of alkaline phosphatase conjugated with TXB₂.
3. **TXB₂ ELISA Antibody, 5 mL, Catalog No. 80-0006**
A yellow solution of a rabbit polyclonal antibody to TXB₂.
4. **Assay Buffer, 27 mL, Catalog No. 80-0010**
Tris buffered saline containing proteins and sodium azide as preservative.
5. **Wash Buffer Concentrate, 27 mL, Catalog No. 80-1286**
Tris buffered saline containing detergents.
6. **Thromboxane B₂ Standard, 0.5 mL, Catalog No. 80-0007**
A solution of TXB₂ in buffer containing 100,000 pg/mL.
7. **pNpp Substrate, 20 mL, Catalog No. 80-0075**
A solution of p-nitrophenyl phosphate in buffer.
8. **Stop Solution, 5 mL, Catalog No. 80-0247**
A solution of trisodium phosphate in water. Keep tightly capped. Caution: Caustic.
9. **TXB₂ Assay Layout Sheet, 1 each, Catalog No. 30-0003**
10. **Plate Sealer, 1 each, Catalog No. 30-0012**



Reagents
require
separate
storage
conditions.

STORAGE

All components of this kit are stable at 4°C until the kit's expiration date.

OTHER MATERIALS NEEDED

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 μ L and 1,000 μ L.
3. Repeater pipets for dispensing 50 and 200 μ L.
4. Disposable beaker for diluting Wash Buffer.
5. Graduated cylinders.
6. A microplate shaker.
7. Adsorbent paper for blotting.
8. Microplate reader capable of reading at 405 nm, preferably with correction at between 570 and 590 nm.

SAMPLE HANDLING

The TXB₂ enzyme-linked immunosorbent assay is compatible with TXB₂ samples in a wide range of matrices. Samples diluted sufficiently into Assay Buffer can be read directly from the standard curve. Please refer to the Sample Recovery recommendations on page 15 for details of suggested dilutions. However, 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 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 TXB₂ in the appropriate matrix. For tissue, urine and plasma 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 and plasma samples. Urine samples may be used in the assay after dilution in Assay Buffer. Some samples may require extraction for accurate measurement. A suitable extraction procedure is outlined below:

Materials Needed

1. TXB₂ Standard to allow extraction efficiency to be accurately determined.
2. 2 M hydrochloric acid, deionized water, ethanol, hexane and ethyl acetate.
3. 200 mg C₁₈ Reverse Phase Extraction Columns.

PROCEDURE

1. Acidify the plasma, urine or tissue homogenate by addition of 2 M HCl to pH of 3.5. Approximately 50 μ L of HCl will be needed per ml of plasma. Allow to sit at 4°C 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 >95% ethanol followed by 10mL 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 μ L of Assay Buffer to the dried samples. Vortex well then allow to sit for five minutes. Repeat twice more. If analysis is to be delayed, store samples as the eluted ethyl acetate solutions at -80°C 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 12 and 13 for details of extraction protocols.

PROCEDURAL NOTES

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or plastic tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. 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°C in the sealed bag provided. The wells should be used in the frame provided.
8. 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.
9. Prior to the addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.

REAGENT PREPARATION

1. TXB₂ Standard

Allow the 100,000 pg/mL TXB₂ standard solution to warm to room temperature. Label seven 12 x 75 mm glass tubes #1 through #7. Pipet 1,000 µL of standard diluent (Assay Buffer or Tissue Culture Media) into tube #1. Pipet 666 µL of Standard diluent into tubes #2 through #7. Remove 100 µL of diluent from tube #1. Add 100µL of the 100,000 pg/mL standard to tube #1. Vortex thoroughly. Add 333 µL of tube #1 to tube #2 and vortex thoroughly. Add 333 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through #7. The concentration of TXB₂ in tubes #1 through #7 will be 10,000, 3,333, 1,111, 370, 123, 41.1, and 13.7 pg/mL respectively. See TXB₂ Assay Layout Sheet for dilution details. Diluted Standard should be used within 60 minutes of preparation.

2. Wash Buffer

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

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°C.
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 #7 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 Solution 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 μ L 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 dry on a lint free paper towel to remove any remaining wash buffer.
11. Add 5 μ L of the blue Conjugate to the TA wells.
12. Add 200 μ L of the pNpp Substrate solution to every well. Incubate at room temperature for 45 minutes without shaking.
13. Add 50 μ L 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.

CALCULATION OF RESULTS

Several options are available for the calculation of the concentration of 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 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:

$$\text{Average Net OD} = \text{Average OD} - \text{NSB OD}$$

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

$$\text{Percent Bound} = (\text{Net OD} / \text{Net Bo OD}) \times 100$$

3. Plot Percent Bound versus Concentration of TXB₂ for the standards. Approximate a straight line through the points. The concentration of TXB₂ in the unknowns can be determined by interpolation.

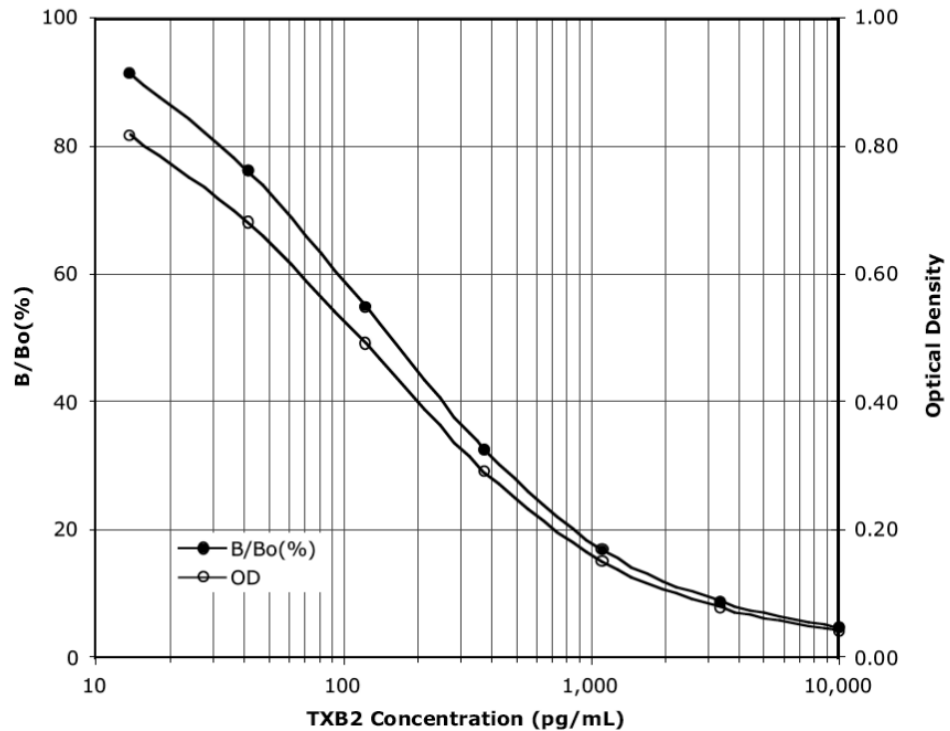
TYPICAL RESULTS

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

Sample	Mean OD (- Blank)	Average Net OD	Percent Bound	TXB ₂ (pg/mL)
Blank OD	(0.123)			
TA	0.693	0.570		
NSB	-0.003	0.000		
Bo	0.891	0.894	100%	0
S1	0.038	0.041	4.6%	10,000
S2	0.075	0.078	8.7%	3,333
S3	0.147	0.150	16.8%	1,111
S4	0.288	0.291	32.4%	370.3
S5	0.488	0.491	54.9%	123.4
S6	0.678	0.681	76.2%	41.15
S7	0.814	0.817	91.3%	13.7
Unknown 1	0.154	0.157		1,052
Unknown 2	0.410	0.413		184

Typical Standard Curves

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



Typical Quality Control Parameters

Total Activity Added	=	0.570 x 10 = 5.7
%NSB	=	-0.055%
%Bo/TA	=	15.7%
Quality of Fit	=	1.000 (Calculated from 4 parameter logistic curve fit)
20% Intercept	=	849 pg/mL
50% Intercept	=	153 pg/mL
80% Intercept	=	33 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 Protocols¹⁴.

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 #7. The detection limit was determined as the concentration of TXB₂ measured at two (2) standard deviations from the zero along the standard curve.

Average Optical Density for the Bo = 0.613 ± 0.020 (2.68%)

Average Optical Density for Standard #7 = 0.561 ± 0.017 (2.46%)

Delta Optical Density (0-13.7 pg/mL) = $0.613 - 0.561 = 0.052$

2 SD's of the Zero Standard = $2 \times 0.020 = 0.040$

Sensitivity = $\frac{0.040}{0.052} \times 13.7 \text{ pg/mL} = 10.54 \text{ pg/mL}$

Linearity

A sample containing 3,239 pg/mL TXB₂ was diluted serially 1:2 seven times with kit Assay Buffer and measured in the assay. The data was plotted graphically as actual TXB₂ Concentration versus measured TXB₂ Concentration.

The line obtained had a slope of 1.055 with a correlation coefficient of 0.999

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of TXB₂ and running these samples multiple times (n=24) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of TXB₂ in multiple assays (n=8).

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

	TXB2 (pg/mL)	Intra-assay %CV	Inter-assay %CV
Low	759	3.6	
Medium	1,283	4.0	
High	2,605	1.6	
Low	44		7.6
Medium	371		3.6
High	3,053		6.2

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 500,000 to 13.7 pg/mL. These samples were then measured in the TXB₂ assay, and the measured TXB₂ concentration at 50% B/B₀ 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
TXB ₂	100%
2,3-dinor TXB ₂	7.1%
11-dehydro TXB ₂	0.4%
6-keto-PGF1 α	0.23%
PGD ₂	<0.01%
PGE ₂	<0.01%
PGF1 α	<0.01%
PGF2 α	<0.01%

SAMPLE RECOVERIES

Please refer to pages 6-9 for Sample Handling recommendations and Standard preparation.

TXB₂ concentrations were measured in a variety of different samples, including tissue culture media, human saliva, serum, plasma and urine. For tissue culture media, saliva and urine samples TXB₂ was spiked into the undiluted samples, which were then diluted with the kit Assay Buffer. For human serum and plasma the endogenous levels of TXB₂ in the samples were used. The serum and plasma samples were diluted into the kit Assay Buffer and then assayed in the kit. The following results were obtained:

Sample	% Recovery*	Recommended Dilution*
Tissue Culture Media	104.7	None
Human Saliva	98.3	1:10
Human Urine	102.2	1:10-1:100
Human Heparin Plasma	111.0	>1:100
Human Serum	113.0	>1:100

* See Sample Handling instructions on page 6 for details.

REFERENCES

1. T. Chard, "An Introduction to Radioimmunoassay & Related Techniques 4th Ed.", (1990) Amsterdam: Elsevier.
2. P. Tijssen, "Practice & Theory of Enzyme-linked immunosorbent assays", (1985) Amsterdam: Elsevier.
3. S. Moncada et al., *Pharm. Rev.*, (1978) 30: 293.
4. H. Sinzinger, et al., *Eicosanoids*, (1990) 3: 59.
5. É.F. Smith III, *Eicosanoids*, (1989) 2: 199.
6. M.D. Mitchell, et al., *J. Endocrinol.*, (1978) 78: 435-441.
7. L. Viinikka & O. Ylikorkala, *Prostaglandins*, (1980) 20: 759-766.
8. E.K. Jackson, et al., *Arch. Pharmacol.*, (1988) 337: 183.
9. S. Conti, et al., *J. Pharm. Pharmacol.*, (1988) 40: 144.
10. K.D. Butler, et al., *Thrombosis Res.*, (1987) 45: 751.
11. G.A. FitzGerald, et al., *Adv. Prostaglandin & Thromboxane Res.*, (1985) 15: 87.
12. D.A. Weerasekera, et al., *Adv. Prostaglandin & Thromboxane Res.*, (1983) 11: 185-190.
13. H.R. Schroeder, et al., *Meth. Enzymol.*, (1978) 57: 424-445.
14. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.



Product Manual

NOTES



Product Manual

www.enzolifesciences.com

Enabling Discovery in Life Science®

GLOBAL HEADQUARTERS

Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free: 1.800.942.0430
Phone: 631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA

Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone: +41/0 61 926 89 89
Fax: +41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:

www.enzolifesciences.com