LSBio™ Human F2 / Prothrombin / Thrombin ELISA Kit

Catalog No. LS-F10429

User Manual

Please Read the Manual Carefully
Before Starting your Experiment

LSBio
LifeSpan BioSciences, Inc.

For research use only. Not approved for use in humans or for clinical diagnosis.

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Strip well format. Reagents for up to 96 tests.

Human Prothrombin

INTENDED USE

This human prothrombin antigen assay is intended for the quantitative determination of total prothrombin antigen in human plasma. **For research use only.**

BACKGROUND

Prothrombin (aka Factor II) is a single-chain vitamin K-dependent 579 amino acid glycoprotein zymogen [1]. Prothrombin is proteolytically activated to thrombin by the prothrombinase enzyme complex in the coagulation cascade common pathway. The serine protease thrombin converts plasma fibrinogen to insoluble fibrin. Prothrombin levels are decreased by anticoagulant therapy, vitamin K deficiency and severe liver disease [2]. Elevated plasma prothrombin is associated with a single nucleotide change at position 20210 [3].

ASSAY PRINCIPLE

Human prothrombin will bind to the capture antibody coated on the microtiter plate. Prothrombin, thrombin, and thrombin-antithrombin complex will react with the antibody on the plate. After appropriate washing steps, anti-human prothrombin primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with the secondary antibody. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human prothrombin. Color development is proportional to the concentration of total prothrombin in the samples.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human prothrombin antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human prothrombin standard:** 1 vial lyophilized standard
- **Anti-human prothrombin primary antibody:** 1 vial lyophilized polyclonal antibody
- **Anti-rabbit horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1000ng/ml standard solution.

Dilution table for preparation of human prothrombin standard:

Prothrombin concentration (ng/ml)	Dilutions
100	900µl (BB) + 100µl (from vial)
50	500µl (BB) + 500µl (100ng/ml)
25	500µl (BB) + 500µl (50ng/ml)
10	600µl (BB) + 400µl (25ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2.5	500µl (BB) + 500µl (5ng/ml)
1	600µl (BB) + 400µl (2.5ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.25	500µl (BB) + 500µl (0.5ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl prothrombin standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures human prothrombin antigen in the 0.25-100 ng/ml range. If the unknown is thought to have high prothrombin levels, dilutions may be made in blocking buffer. A 1:5,000-1:40,000 dilution for normal human plasma is suggested for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 2µl of conjugated secondary antibody in 10ml of blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

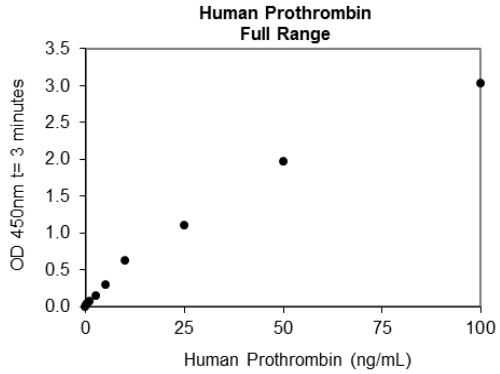
Measurement

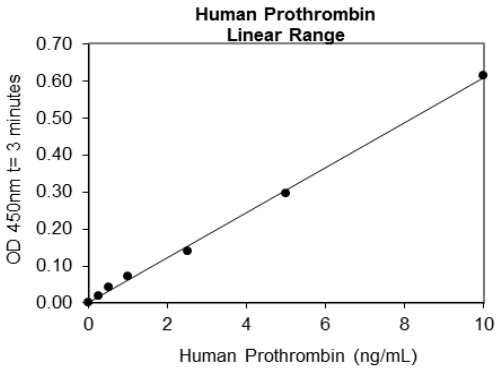
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of prothrombin in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of prothrombin in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):





EXPECTED VALUES

Prothrombin in normal human plasma ranges from 110-212 µg/ml with an average concentration of 150 µg/ml [4].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.147-0.169) and calculating the corresponding concentration. The MDD was 0.166 ng/ml.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	2.58	10.2	38.8
Standard Deviation	0.174	0.978	2.96
CV (%)	6.77	9.55	7.63

Inter-assay Precision: Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2	3
n	10	10	10
Mean (ng/ml)	2.33	9.44	44.2
Standard Deviation	0.283	0.430	4.42
CV (%)	12.1	4.55	10.0

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	2.17	7.23	19.6	66.5
Average % Recovery	109	103	98	111
Range	99-120%	92-114%	85-115%	93-119%

Linearity: To assess the linearity of the assay, pooled citrated human plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	107	105	97	126
Range	85-118%	102-109%	93-99%	105-151%

Specificity: This assay recognizes natural human total prothrombin (prothrombin, thrombin, and thrombin-antithrombin complex). Pooled normal plasma from mouse, pig, and canine were assayed for cross-reactivity. No significant cross-reactivity was observed.

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Mann KG: Methods Enzymol. 1976, 45(Pt B):123-156.
2. A.D.A.M. Medical Encyclopedia [Internet]. Atlanta (GA): A.D.A.M., Inc.; c1997-2011. Factor II deficiency; [last reviewed 2011 Feb 28; cited 2012].
3. Poort SR *et al.*: Blood. 1996, 88:3698-3703.
4. McDuffie FC *et al.*: Thromb Res. 1979, 16:759-773.

Example of ELISA Plate Layout
96 Well Plate: 20 Standard wells, 76 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	100 ng/ml		
B	0	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	100 ng/ml		
C												
D												
E F												
G												
H												

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion.

Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

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