

# Human Prekallikrein Total Antigen Assay

Strip well format. Reagents for up to 96 tests.

## For Research Use Only.

### INTENDED USE

This human prekallikrein total antigen assay is intended for the quantitative determination of total prekallikrein antigen in human plasma.

### BACKGROUND

Prekallikrein is the glycosylated single chain zymogen precursor of the plasma serine protease kallikrein. Plasma prekallikrein circulates with kininogen and is activated by Factor XIIa in the intrinsic coagulation pathway. Kallikrein activates plasminogen in fibrinolysis and cleaves kininogen in the bradykinin system of vasodilation. Prekallikrein deficiency is rare and causes increased activated partial thromboplastin time [1]. Elevated plasma prekallikrein is associated with diabetes [2] and cardiovascular disease [3].

### ASSAY PRINCIPLE

Human prekallikrein will bind to the capture antibody coated on the microtiter plate. After appropriate washing steps, affinity purified polyclonal anti-human prekallikrein primary antibody binds to the captured protein. Excess antibody is washed away and bound polyclonal antibody is reacted with secondary antibody conjugated to horseradish peroxidase. 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 prekallikrein. Color development is proportional to the total concentration of prekallikrein in the samples.

### REAGENTS PROVIDED

#### ◆Immunoassay plate:

1-96 well immulon plate (8X12 removable wells) coated, blocked, and dried with prekallikrein capture antibody

#### ◆10X Wash Buffer:

1 bottle of 50ml; bring to 1X using DI water

#### ◆Human prekallikrein antigen standard:

1 vial of lyophilized standard

#### ◆Anti-human prekallikrein primary antibody:

1 vial of lyophilized polyclonal antibody

#### ◆Avidin peroxidase secondary antibody:

1 vial of concentrated HRP labeled antibody

#### ◆TMB substrate solution:

1 bottle of 10ml solution

### STORAGE AND STABILITY

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

### REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes, 1.5ml centrifuge tubes
- 1N H<sub>2</sub>SO<sub>4</sub>
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450nm

•Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

#### WARNINGS

**Warning** – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

#### PRECAUTIONS

- 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.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **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:** 3% BSA in TBS buffer

#### SPECIMEN COLLECTION

The assay measures total human prekallikrein in the 0.02-10µg/ml range. Samples giving prekallikrein levels above 20µg/ml should be diluted in blocking buffer before use.

#### ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay. For plasma, a 1:2 dilution in blocking buffer is suggested for best results.

#### Preparation of Standard:

Reconstitute standard as directed on the vial to give a 10µg/ml solution.

Dilution table for preparation of human prekallikrein standards:

Prekallikrein concentration (µg/ml)	Dilutions
10	Straight from vial
5	500µl (Blocking buffer) + 500µl (10µg/ml)
2	600µl (Blocking Buffer) + 400µl (5µg/ml)
1	500µl (Blocking Buffer) + 500µl (2µg/ml)
0.5	500µl (Blocking Buffer) + 500µl (1µg/ml)
0.2	600µl (Blocking Buffer) + 400µl (0.5µg/ml)
0.1	500µl (Blocking Buffer) + 500µl (0.2µg/ml)
0.05	500µl (Blocking Buffer) + 500µl (0.1µg/ml)
0.02	600µl (Blocking Buffer) + 400µl (0.05µg/ml)
0	500µl (Blocking Buffer) Zero point to determine background

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

#### Standard and Unknown Addition:

Remove microtiter plate from bag. Add 100µl 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.

#### Primary Antibody Addition:

Add 10ml of blocking buffer directly to the primary antibody 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:**

Dilute 2µl into 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 of TMB substrate solution to all wells and shake plate at 300rpm for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H<sub>2</sub>SO<sub>4</sub> 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 and read final absorbance values at 450nm. For best results read plate immediately.

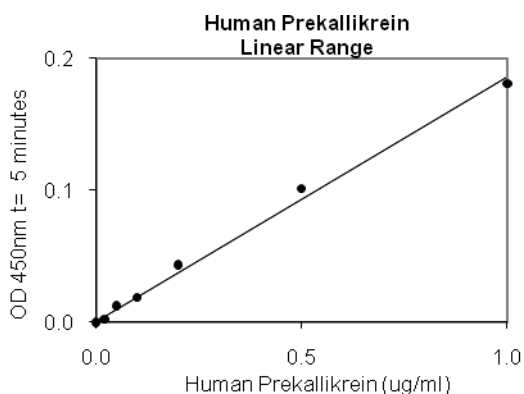
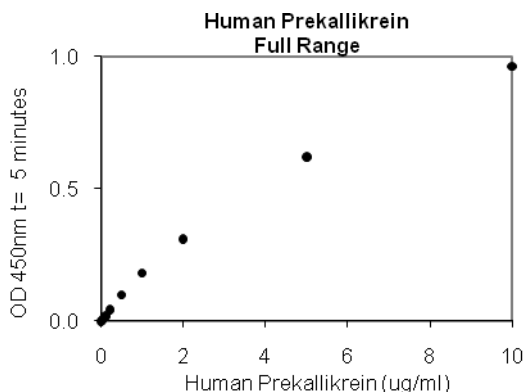
**Measurement:**

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 ( $A_{450}$ ).

**Assay Calibration:**

Plot  $A_{450}$  against the amount of prekallikrein in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total human prekallikrein in the unknowns can be determined from this curve.

A typical standard curve.  
(EXAMPLE ONLY, DO NOT USE)

**EXPECTED VALUES**

Prekallikrein is present in pooled human plasma at concentrations of 15µg/ml [4] – 55µg/ml [5] as determined by coagulation assay. Prekallikrein antigen measured by ELISA has not been reported.

**PERFORMANCE CHARACTERISTICS**

The assay measures total human prekallikrein in the 0.02-10µg/ml range. The typical linear range is 0.02-1µg/ml.

#### 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 or from contact with the above product.

#### REFERENCES

1. DG Sollo and A Saleem: Prekallikrein (Fletcher factor) deficiency. Ann. Clin. Lab. Sci., **15**:279-285, 1985.
2. Jaffa, AA *et al.*: Plasma Prekallikrein. A Risk Marker for Hypertension and Nephropathy in Type 1 Diabetes. Diabetes, **52**:1215-1221, 2003.

3. Mackenzie, JA *et al.*: Plasma prekallikrein levels are positively associated with circulating lipid levels and the metabolic syndrome in children. Appl. Physiol. Nutr. Metab., **35**(4):518-525, 2010.

4. RL Heimark and EW Davie: Bovine and human plasma prekallikrein. Methods Enzymol., **80**(Pt C):157-172, 1981.

5. Bouma, AA *et al.*: Human plasma prekallikrein. Studies of its activation by activated factor XII and of its inactivation by diisopropyl phosphorfluoridate. Biochemistry, **19**(6):1151-1160, 1980.

#### Layout Example of 96 Well Plate

**Standards:** 20 wells

**Samples:** 76 wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.02µg/ml	0.05µg/ml	0.1µg/ml	0.2µg/ml	0.5µg/ml	1µg/ml	2µg/ml	5µg/ml	10µg/ml		
B	0	0.02µg/ml	0.05µg/ml	0.1µg/ml	0.2µg/ml	0.5µg/ml	1µg/ml	2µg/ml	5µg/ml	10µg/ml		
C												
D												
E												
F												
G												
H												