

### INTENDED USE

This mouse prorenin/renin total antigen assay is intended for the quantitative determination of total prorenin and renin antigen in cell culture media samples.

**For research use only.**

### BACKGROUND

Prorenin is a glycosylated aspartic protease that consists of 2 homologous lobes and is the precursor of renin. Renin activates the renin-angiotensin system by cleaving angiotensinogen, produced by the liver, to yield angiotensin I, which is further converted into angiotensin II by ACE, the angiotensin-converting enzyme primarily within the capillaries of the lungs. It has been reported that the levels of circulating prorenin (but not renin) are increased in diabetic subjects [1].

### ASSAY PRINCIPLE

Mouse prorenin and renin will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled polyclonal anti-mouse prorenin primary antibody binds to the captured protein. Excess antibody is washed away and bound polyclonal antibody is reacted with streptavidin 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 mouse prorenin. Color development is proportional to the total concentration of prorenin and renin in the samples.

### REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-mouse prorenin/renin antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Mouse prorenin standard:** 1 vial lyophilized standard
- **Anti-prorenin/renin primary antibody:** 1 vial lyophilized polyclonal antibody
- **Horseradish peroxidase-conjugated streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **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<sub>2</sub>SO<sub>4</sub> 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

## 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 mouse prorenin standard:

Prorenin concentration (ng/ml)	Dilutions
100	900 $\mu$ l (BB) + 100 $\mu$ l (from vial)
50	500 $\mu$ l (BB) + 500 $\mu$ l (100ng/ml)
20	600 $\mu$ l (BB) + 400 $\mu$ l (50ng/ml)
10	500 $\mu$ l (BB) + 500 $\mu$ l (20ng/ml)
5	500 $\mu$ l (BB) + 500 $\mu$ l (10ng/ml)
2	600 $\mu$ l (BB) + 400 $\mu$ l (5ng/ml)
1	500 $\mu$ l (BB) + 500 $\mu$ l (2ng/ml)
0.5	500 $\mu$ l (BB) + 500 $\mu$ l (1ng/ml)
0	500 $\mu$ l (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 100 $\mu$ l prorenin 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 300 $\mu$ l wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures total mouse prorenin and renin antigens in the 0.5-100 ng/ml range. If the unknown is thought to have high prorenin levels, dilutions may be made in blocking buffer.

### Primary Antibody Addition

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

### Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 1 $\mu$ l of HRP conjugated streptavidin into 1 ml blocking buffer to generate a 1:1,000 dilution. Add 0.2ml of 1:1,000 dilution to 9.8ml of blocking buffer to generate a 1:50,000 dilution. Add 100 $\mu$ l of the 1: 50,000 dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu$ l wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### Substrate Incubation

Add 100 $\mu$ l 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 50 $\mu$ l of 1N H<sub>2</sub>SO<sub>4</sub> 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.

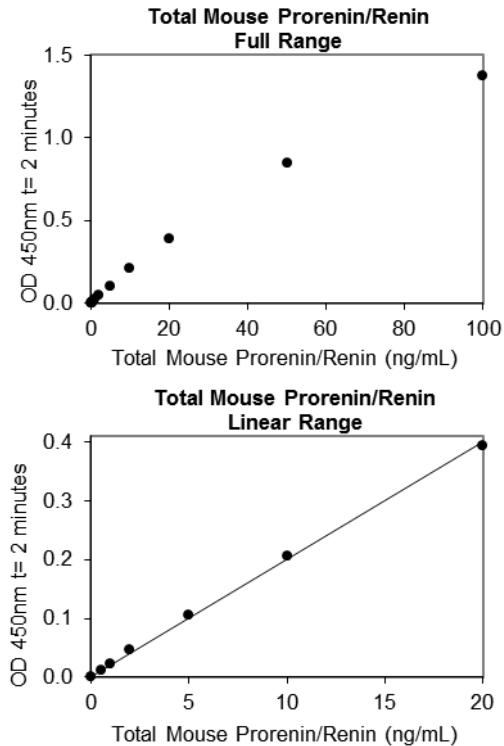
### Measurement

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<sub>450</sub>).

### Calculation of Results

Plot A<sub>450</sub> against the amount of prorenin 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 prorenin 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

Prorenin and renin total antigen levels in mouse plasma range from 20-30 ng/ml [2]. Human plasma levels of prorenin are greater in males than females and correlate positively with age and negatively with blood pressure [3]. Plasma and serum concentrations increase in several conditions such as pregnancy, progressive diabetes mellitus, diabetes mellitus with microvascular disease, and diabetic retinopathy [4,5].

### 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<sub>450</sub>: 0.126-0.137) and calculating the corresponding concentration. The MDD was 0.335ng/ml.

**Intra-assay Precision:** These studies are currently in progress. Please contact us for more information.

**Inter-assay Precision:** These studies are currently in progress. Please contact us for more information.

**Recovery:** These studies are currently in progress. Please contact us for more information.

**Linearity:** These studies are currently in progress. Please contact us for more information.

**Specificity:** These studies are currently in progress. Please contact us for more information.

### 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. Luetscher JA, *et al.*: N Engl J Med. 1985, 312:1412-1417.
2. Bohlender J, *et al.*: Am J Physiol Heart Circ Physiol. 1998, 274:H1450-H1456.
3. Danser AH, *et al.*: J Hypertens. 1988, 16:853-862.
4. Yokota H, *et al.*: Br J Ophthalmol. 2005, 89:871-873.
5. Schmieder RE: J Hypertens. 2007, 25:1323-1326.

Example of ELISA Plate Layout

96 Well Plate: 18 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml			
B	0	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml			
C												
D												
E												
F												
G												
H												

**SAMPLE INSERT**  
**Refer to kit box for**  
**lot specific instructions**