

**Enzymatic Assay of RENIN**  
**(EC 3.4.99.19)**

**PRINCIPLE:**

Angiotensinogen  $\xrightarrow{\text{Renin}}$  Angiotensin + Peptide Fragment

**CONDITIONS:** T = 37°C, pH = 6.0

**METHOD:** Radioimmunoassay<sup>1</sup>

**REAGENTS:**

- A. 0.84% (w/v) Dimercaprol with 1.87% (w/v) Benzyl Benzoate (Dimercaprol Soln)  
(Use Dimercaprol Solution from the DuPont Angiotensin RIA Kit, Dupont Prod. No. NEA-105.)
- B. 3.3% (w/v) 8-Hydroxyquinoline Solution (8-Hydroxyquin)  
(Use 8-Hydroxyquinoline Solution from the DuPont Angiotensin RIA Kit, Dupont Prod. No. NEA-105.)
- C. 2.0% (w/v) Maleate Buffer, pH 6.0 at 25°C  
(Use Maleate Buffer pH 6.0 from the DuPont Angiotensin RIA Kit, Dupont Prod. No. NEA-105.)
- D. 5% (w/v) Bovine Serum Albumin (Enzyme Diluent)  
(Prepare 10 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-7906.)
- E. Angiotensinogen Solution (Angiotensinogen Soln)  
(Immediately before use prepare a solution containing 0.2 unit/ml of Angiotensinogen, Sigma Prod. No. A-2283, in cold deionized water.)
- F. "0" Standard  
( "0" Standard is from the DuPont Angiotensin RIA Kit, Dupont Prod. No. NEA-105.)
- G. Renin Solution  
(Immediately before use prepare a solution containing 0.0001 unit/ml in Reagent D by adding 3 ml of Reagent D to a 1 unit vial of Renin, Sigma Prod. No. R-2761. Continue diluting (3000X) with Reagent D.)

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**REAGENTS:**

- H. Angiotensin Standards (Angiotensin Std)  
(Use Angiotensin Standards from the DuPont Angiotensin RIA Kit, DuPont Prod. No. NEA-105.)
- I. Angiotensin <sup>125</sup>I labelled  
(Use Angiotensin <sup>125</sup>I from the DuPont Angiotensin RIA Kit, DuPont Prod. No. NEA-105.)
- J. Blank Antiserum  
(Use Blank Antiserum from the DuPont Angiotensin RIA Kit, DuPont Prod. No. NEA-105.)
- K. Angiotensin I Antiserum  
(Use Blank Angiotensin I Antiserum from the DuPont Angiotensin RIA Kit, DuPont Prod. No. NEA-105.)
- L. Angiotensin I Second Antibody  
(Use Angiotensin I Second Antibody from the DuPont Angiotensin RIA Kit, DuPont Prod. No. NEA-105.)

**PROCEDURE:**

Step 1:

Pipette (in milliliters) the following reagents in the following order into a 12 X 75 mm polystyrene tube:

Reagent G (Enzyme Solution)	0.500
Reagent A (Dimercaprol Soln)	0.005
Reagent B (8-Hydroxyquin)	0.005
Reagent E (Angiotensinogen Soln)	0.100
Reagent C (Maleate Buffer)	1.000

Vortex the tube for 3 seconds, then immediately pipette 0.1 ml of this solution into a suitable tube labeled "Cold Mix" and store in an ice bath. Incubate the remainder of the reaction mix at 37°C in a suitable water bath.

Pipette

0.1 ml of this reaction mix at the end of 10 minutes, 20 minutes, and 30 minutes, into suitable tubes labeled "10 minutes", "20 minutes", "30 minutes", respectively and store in a ice bath.

Step 2:

Pipette (in milliliters) the following reagents in the order listed below into suitable 12 X 75 mm polystyrene tubes using reagents supplied with the kit.

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**PROCEDURE:** (continued)

Tube Number	"O" Std Reagent F	Angiotensin Std Reagent H	Samples	Angiotensin (I <sup>125</sup> ) Reagent I	Blank Antiserum Reagent J	Angiotensin I Antiserum Reagent K
Total Counts 1				0.1 ml		
Total Counts 2				0.1 ml		
Blanks 3	0.1 ml			0.1 ml	0.1 ml	
Blanks 4	0.1 ml			0.1 ml	0.1 ml	
"O" Std 5	0.1 ml			0.1 ml		0.1 ml
"O" Std 6	0.1 ml			0.1 ml		0.1 ml
0.01 ng 7		0.1 ml		0.1 ml		0.1 ml
0.01 ng 8		0.1 ml		0.1 ml		0.1 ml
0.025 ng 9		0.1 ml		0.1 ml		0.1 ml
0.025 ng 10		0.1 ml		0.1 ml		0.1 ml
0.05 ng 11		0.1 ml		0.1 ml		0.1 ml
0.05 ng 12		0.1 ml		0.1 ml		0.1 ml
0.10 ng 13		0.1 ml		0.1 ml		0.1 ml
0.10 ng 14		0.1 ml		0.1 ml		0.1 ml
0.25 ng 15		0.1 ml		0.1 ml		0.1 ml
0.25 ng 16		0.1 ml		0.1 ml		0.1 ml
0.50 ng 17		0.1 ml		0.1 ml		0.1 ml
0.50 ng 18		0.1 ml		0.1 ml		0.1 ml
1.00 ng 19		0.1 ml		0.1 ml		0.1 ml
1.00 ng 20		0.1 ml		0.1 ml		0.1 ml
Samples						
10 min 21			0.1 ml	0.1 ml		0.1 ml
20 min 22			0.1 ml	0.1 ml		0.1 ml
30 min 23			0.1 ml	0.1 ml		0.1 ml
Cold Mix Sample 24			0.1 ml	0.1 ml		0.1 ml

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**PROCEDURE:** (continued)

Once all the reagents have been added to the appropriate tubes, vortex each tube for 5 seconds and incubate for two hours at room temperature.

At the end of the two hour incubation, mix the Angiotensin I Second Antibody (Reagent L) well and pipette 0.5 ml of Reagent L into each tube except in the tubes labelled "Total Counts." Vortex each tube and incubate for 20 - 30 minutes at room temperature.

Centrifuge all tubes at 1000 X g for 15 minutes. Remove the supernatant from each tube (except the tubes labelled "Total Counts") by aspiration into a <sup>125</sup>I liquid waste container.

Cap and count each tube in a gamma counter for one minute per tube.

**CALCULATIONS:**

Determine the ng Angiotensin I of each reacted sample tube and cold mix sample tube of each set from the standard curve. The standard curve is generated from a plot of percentage bound of each standard relative to that bound for the "0" standard (%B/B<sub>0</sub>) versus ng angiotensin I of the standard.

$$\%B/B_0 = (\text{Net counts for standard} / \text{Net counts for "0" standard}) \times 100$$

If the gamma counter is not equipped with a data reproduction unit which will generate a standard curve and final sample results, it will be necessary to plot the standard curve on semi-log graph paper from which the sample ng amount of angiotensin I levels can be obtained.

$$\text{Units/ml} = \frac{(\text{ng Sample} - \text{ng Cold Mix}) (1.61) (1000)}{(0.5) (0.1) (\text{Time}) (100,000)}$$

1.61 = Final reaction volume in ml  
3000 = Dilution factor for the Renin  
0.5 = Volume of Renin sample added to reaction mix in ml  
0.1 = Volume of reaction mix removed in ml  
Time = Hours expressed in fractions  
100,000 = Conversion of DuPont to Sigma Units

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**UNIT DEFINITION:**

One unit will liberate 100 µg of angiotensin I from angiotensinogen (A2283) per hour at pH 6.0 at 37°C.

**FINAL ASSAY CONCENTRATION:**

In a 1.61 ml reaction mixture the final concentrations are 0.003% (w/v) dimercaprol, 0.006% (w/v) benzyl benzoate, 0.01% (w/v) 8-hydroxyquinoline, 1.2% (w/v) maleate, 0.02 unit angiotensinogen, 2% (w/v) bovine serum albumin, and  $3 \times 10^{-5}$  unit renin.

**REFERENCES:**

Dupont (1991) *Angiotensin I [<sup>125</sup>I] Radioimmunoassay Kit*, NEA-104, 105 Instruction Manual

**NOTES:**

1. The amount of generated angiotensin I is measured by radioimmunoassay. The basic principle of this procedure is the competition between radioactive and non-radioactive antigen for a fixed number of antibody binding sites. If increasing amounts of non-radioactive antigen (i.e., standards or unknowns) and a fixed amount of tracer are allowed to react with a constant and limiting amount of antibody, a decreasing quantity of radioactive antigen is bound to the antibody. The separation of bound from free antigen is achieved by the addition of a second antibody which precipitates the primary antigen-antibody complex. Polyethylene glycol is used to accelerate this reaction. The second antibody is prepared against gamma globulin of the species used for raising the primary antibody. After incubation and centrifugation, the supernatant is discarded and the antigen-antibody complex is counted to quantitate the bound tracer. The data are used to construct a standard curve from which the values of the unknowns may be obtained by interpolation. Plasma renin is expressed as ng/ml/hr of angiotensin I generated.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**