Product Manual

# Plasminogen (human) ELISA kit

Catalog # ADI-900-231
96-Well Kit

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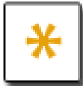
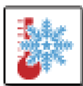
# Product Manual



Please read entire booklet before proceeding with the assay.

Please contact Enzo Life Sciences Technical Support if necessary.



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

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## INTRODUCTION

Plasminogen, a 92kDa glycoprotein, is produced by the liver and is present in plasma and extracellular fluids. Plasminogen is the inactive precursor of plasmin, a potent serine protease involved in the dissolution of fibrin blood clots. Plasminogen can be converted into the active plasmin by plasminogen activators urokinase (uPA), tissue plasminogen activator (tPA), factor XII-dependent components. The plasmin system has been implicated in a variety of physiological and pathological processes such as fibrinolysis, tissue remodeling, cell migration, inflammation, and tumor invasion and metastasis. Hereditary defects of plasminogen are a predisposing risk factor for thromboembolic disease.

## PRINCIPLES OF METHOD

The design of this assay is based on a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to human plasminogen. Samples are pipetted into these wells. Nonbound plasminogen and other components of the sample should be removed by washing, then biotin-conjugated monoclonal antibody specific to plasminogen added. In order to quantitatively determine the amount of plasminogen present in the sample, Avidin conjugated to Horseradish Peroxidase (HRP) should be added to each microplate well. The final step, a TMB-substrate solution added to each well. Finally, a sulfuric acid solution is added and the resulting yellow colored product is measured at 450nm. Since the increases in absorbency is directly proportional to the amount of captured plasminogen.

## INTENDED USE

The Enzo Life Sciences Plasminogen (human) ELISA kit is to be used for the *in vitro* quantitative determination of human plasminogen in human serum, human plasma, cell lysate and buffered solution. The assay will recognize native human plasminogen.

**This kit has been configured for research use only and is not to be used in diagnostic procedures.**

## STORAGE AND STABILITY

All kit components of this kit are stable at 2-8°C. Any unused reconstituted standard should be discarded or frozen at -70°C. **Standard can be frozen and thawed one time only without loss of immunoreactivity.**

## CHEMICAL HAZARD

**Stop solution**: This reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection. In the event of contact with eyes or skin, wash immediately with plenty of water.

**Standard protein** and **2nd Antibody** containing Sodium Azide as a preservative.

## KIT CONTENTS

| Contents | Number | Volume |
|---|---|---|
| **96 Well Plate** | 1 (in aluminum foil bag with desiccant) | |
| **Incubation Buffer** | 1 | 30ml |
| **Washing Buffer** | 2 | (20X) 25ml |
| **Standard Protein** | 1 Glass vial (lyophilized) | |
| **Standard/Sample Dilution Buffer** | 1 | 25ml |
| **Secondary Antibody** | 1 Glass vial (lyophilized) | |
| **AV-HRP** | 1 | 150µl |
| **Secondary Antibody/ AV-HRP Dilution Buffer** | 1 | 25ml |
| **Substrate (TMB)** | 1 | 15ml |
| **Stop Solution** | 1 | 15ml |
| **Protocol booklet** | 1 | - |
| **Plate sealers** | 2 | - |

1. **96 Well Plate**:
   Human plasminogen microtiter plate, one plate of 96 wells

   A plate using break-apart strips coated with a mouse monoclonal antibody specific to human plasminogen.
2. **Standard Protein**:
   Native human plasminogen.
3. **Secondary Antibody**:
   Biotinylated anti human plasminogen.
4. **AV-HRP**
   Avidin linked Horseradish Peroxidase (HRP, enzyme)
5. **Substrate** (Stabilized chromogen)
   Tetramethylbenzidine (TMB) solution
6. **Stop Solution**
   1N solution of sulfuric acid ($H_2SO_4$).
7. **Plate sealer**
   Adhesive sheet.

*Do not mix or interchange different reagents from various kit lots.*

## OTHER MATERIALS NEEDED

1. Microtiter plate reader capable of measurement at or near 450nm.
2. Calibrated, adjustable precision pipettes, preferably with disposable plastic tips (A manifold multi-channel pipette is desirable for large assays.)
3. Distilled or deionized water
4. Data analysis and graphing software
5. Vortex mixer
6. Polypropylene tubes for diluting and aliquoting standard
7. Absorbent paper towels
8. Calibrated beakers and graduated cylinders of various sizes

## REAGENT PREPARATION

### Human Plasminogen standard

1. Reconstitute the lyophilized Human Plasminogen standard by adding 1ml of **Standard/Sample Dilution Buffer** to make the 0.2µg/ml standard stock solution. Allow solution to sit at RT for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstituting. Standard can be frozen and thawed one time only without loss of immunoreactivity.
2. Prepare 1ml of 64ng/ml top standard by adding 320µl of the above stock solution in 750µl of **Standard/Sample Dilution Buffer**. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay (1ng/ml ~ 64ng/ml) as below. **Standard/Sample Dilution Buffer** serves as the zero standard (0ng/ml).

| Standard | Add | Into |
|---|---|---|
| 64ng/ml | 320.0µl of the std. (0.2µg/ml) | 680.0µl of the Standard/ Sample Dilution Buffer |
| 32ng/ml | 500µl of the std. (64ng/ml) | 500.0µl of the Standard/ Sample Dilution Buffer |
| 16ng/ml | 500µl of the std. (32ng/ml) | 500.0µl of the Standard/ Sample Dilution Buffer |
| 8ng/ml | 500µl of the std. (16ng/ml) | 500.0µl of the Standard/ Sample Dilution Buffer |
| 4ng/ml | 500µl of the std. (8ng/ml) | 500.0µl of the Standard/ Sample Dilution Buffer |
| 2ng/ml | 500µl of the std. (4ng/ml) | 500.0µl of the Standard/ Sample Dilution Buffer |
| 1ng/ml | 500µl of the std. (2ng/ml) | 500.0µl of the Standard/ Sample Dilution Buffer |
| 0ng/ml | 1.0ml of the Standard/Sample Dilution Buffer | |

### Secondary Antibody

100X secondary antibody solution can be made by adding 150µl secondary antibody/AV-HRP dilution buffer in the vial.

1. Equilibrate to room temperature, mix gently.
2. Mix 20µl **Secondary Antibody concentrated solution** (100X) + 1.98ml **Secondary Antibody/AV-HRP dilution buffer**.

   Label as “*Working Secondary antibody Solution*”.
3. Return the unused **Secondary Antibody concentrated solution** to the refrigerator.

**AV-HRP**

1. Equilibrate to room temperature, mix gently.
2. Mix 20µl **AV-HRP concentrated solution** (100X) + 1.98ml **Secondary Antibody/AV-HRP dilution buffer**.
3. Label as "Working AV-HRP Solution".
4. Return the unused **AV-HRP concentrated solution** to the refrigerator.

**Washing buffer**

1. Equilibrate to room temperature, mix to re-dissolve any precipitated salt.
2. Mix 0.5 volume **Wash buffer concentrate solution** (20X) + 9.5 volumes of deionized water. Label as "*Working Washing Solution*".
3. Store both the concentrated and the Working Washing Solution in the refrigerator.

***Directions for washing**

1. Fill the wells with 300µl of "Working Washing Buffer". Let soak for 1 to 3 minutes and then all residual wash-liquid must be drained from the wells by aspiration (taking care not to scratch the inside of the well) or decantation, followed by forceful tapping of the plate on absorbent paper. Never insert absorbent paper directly into the wells.

   If using an automated washer, the operating instructions for washing equipment should be carefully followed.
2. Incomplete washing will adversary affects the assay and renders false results.
3. It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing to avoid strips coming free of the frame.

## SAMPLE PREPARATION

Blood should be collected by veinpuncture. For plasma samples, blood may be drawn into tubes containing sodium citrate or heparin, EDTA. The serum or plasma should be separated from the coagulated or packed cells by centrifugation. Specimens may be shipped at room temperature and then stored refrigerated at 2-8°C if testing is to take place within one week after collection. If testing is to take place later than one week, specimens should be stored at -20°C. Avoid repeated freeze/thawing. Serum and plasma require at least 2000 fold dilution in the Standard/Sample Dilution Buffer.

**Assay Procedure**

- Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.
- All standards, controls and samples should be run in duplicate for confirmation of reproducibility.
- A standard curve must be run with each assay.
- If particulate matter is present in the analyte, centrifuge or filter prior to analysis.
- Maintain a consistent order of components and reagents addition from well to well. This ensures equal incubation times for all wells.

1. Determine the number of strips needed for assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Refrigerate for further use.)
2. Add 300µl of Incubation buffer to all wells and incubate the plate for 5 minutes at room temperature.
3. Thoroughly aspirate or decant the solution from the wells. Wash wells 2 times (See "*Directions for washing*").
4. For the standard curve, add 100µl of the standard to the appropriate microtiter wells. Add 100µl of the **Standard/Sample Dilution Buffer** to zero wells.
5. Serum and plasma require at least 2000 fold dilution in the **Standard/Sample Dilution Buffer**. And add 100µl of sample to each well.
6. Cover the plate with the plate cover and incubate for 2 hours at room temperature.
7. Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "*Directions for washing*").
8. Pipette 100µl of "Working Secondary Antibody Solution" into each well.

9. Cover the plate with the plate cover and incubate for 1 hour at room temperature.
10. Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "*Directions for washing*").
11. Add 100µl "Working AV-HRP Solution" to each well.
12. Cover the plate with the plate cover and incubate for 30 minutes at room temperature.
13. Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "*Directions for washing*").
14. Add 100µl of **Substrate** to each well. The liquid in the wells should begin to turn blue.
15. Incubate the plate at room temperature.
    - Do not cover the plate with aluminum foil, or color may develop.

      *The incubation time for chromogen substrate is often determined by the microtiter plate reader used. O.D. values should be monitored and the substrate reaction stopped before O.D. of the positive wells exceeds the limits of the instrument. O.D. values at 450nm can only be read after the Stop Solution has been added to each well.*
    - Because the **Substrate** is light sensitive, avoid prolonged exposure to light for the remaining Substrate solution.
    - Typically, reaction is stopped 5~10 minutes after treatment of Substrate, but this time can be adjusted as the user desires.
16. Add 100µl of **Stop Solution** to each well. The solution in the wells should change from blue to yellow.
17. Read the absorbance of each well at 450nm. Read the plate within 20 minutes of adding the Stop Solution.
18. Plot the absorbance of the standard against the standard concentration (Optimally, the background absorbance can be subtracted from all data points, including standards, unknowns and controls, prior to plotting.). Draw a smooth curve through these points to construct the standard curve.
19. Read the human plasminogen concentrations for the unknown samples and controls from the standard curve plotted in step 18. Multiply value(s) obtained for the unknown sample by the dilution factor (Samples producing signals greater than that of the highest standard should be further diluted in the **Standard/Sample Dilution Buffer**).

## CHARACTERISTICS

**Typical results**

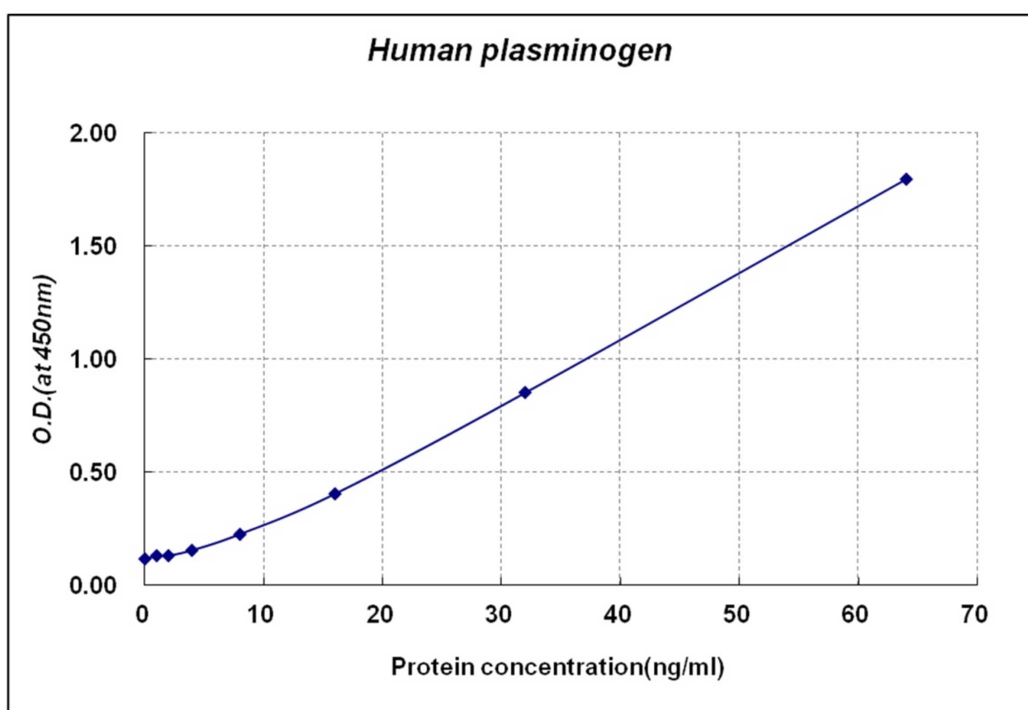
The standard curve below is for illustration only and should not be used to calculate results in your assay.

*A standard curve must be run with each assay.*

| Standard Human Plasminogen (ng/ml) | Optical Density (at 450nm) |
|---|---|
| 0 | 0.116 |
| 1 | 0.129 |
| 2 | 0.131 |
| 4 | 0.155 |
| 8 | 0.226 |
| 16 | 0.405 |
| 32 | 0.853 |
| 64 | 1.798 |

Limitations

- Do not extrapolate the standard curve beyond the 64ng/ml standard point.
- Other buffers and matrices have not been investigated.
- The rate of degradation of native human plasminogen in various matrices has not been investigated.



### Sensitivity

The minimal detectable dose of human plasminogen was calculated to be 2.01ng/ml, by subtracting two standard deviations from the mean of 12 zero standard replicates (ELISA buffer, S0) and intersecting this value with the standard curve obtained in the same calculation.

| N | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ZERO | 0.083 | 0.067 | 0.069 | 0.068 | 0.069 | 0.069 | 0.083 | 0.067 | 0.069 | 0.068 | 0.069 | 0.069 |

| Average | SD | LLD | LLD mean (ng/ml) |
|---|---|---|---|
| 0.0708 | 0.0057 | 0.0823 | 2.0136 |

### Specificity

The following substances were tested and found to have no cross-reactivity: human serum albumin, transferrin, IgG, alpha-fetoprotein (AFP), fibrinogen, Vitamin D binding protein (VDBP), Hemoglobin.

### Precision

1. Within-Run (Intra-Assay)

(n=6)

| Mean (ng/ml) | SD (ng/ml) | CV (%) |
|---|---|---|
| 6.23 | 0.59 | 9.47 |
| 15.05 | 0.64 | 4.27 |
| 33.36 | 0.91 | 2.73 |
| 63.91 | 1.56 | 2.44 |

2. Between-Run (Inter-Assay)

(n=4)

| Mean (ng/ml) | SD (ng/ml) | CV (%) |
|---|---|---|
| 6.52 | 0.14 | 2.18 |
| 15.29 | 0.61 | 4.00 |
| 34.0 | 0.60 | 1.78 |
| 63.50 | 0.23 | 0.37 |

**Recovery**

Recovery on addition is 97.12~103.00% (mean 100.14%).

- Plasminogen in Serum A: 102.177µg/ml;
- Plasminogen in Serum B: 114.834µg/ml

| Analyte added (ng/ml) | Added analyte+1/256000 diluted Serum A (ng/ml) | Recovery (%) | Added analyte+1/256000 diluted Serum B (ng/ml) | Recovery (%) |
|---|---|---|---|---|
| 8 | 8.399 | 103.0 | 8.495 | 101.10 |
| 16 | 16.399 | 100.12 | 16.495 | 99.15 |
| 32 | 32.399 | 97.17 | 32.495 | 100.56 |
| 64 | 64.399 | 102.60 | 64.495 | 97.42 |

For the recovery assay, a known amount of the ELISA standard is spiked into serially-diluted normal human serum and run in the assay. Any endogenous levels are taken into account and then % recovery of the exogenously spike standard is determined.

## TROUBLESHOOTING

| Problem | Possible Cause | Solution |
|---|---|---|
| High signal and background in all wells | Insufficient washing | Increase number of washes<br>Increase time of soaking between in wash |
| | Too much AV-HRP | Check dilution, titration |
| | Incubation time too long | Reduce incubation time |
| | Development time too long | Decrease the incubation time before the stop solution is added |
| No signal | Reagent added in incorrect order, or incorrectly prepared | Review protocol |
| | Standard has gone bad (If there is a signal in the sample wells) | Check the condition of stored standard |
| | Assay was conducted from an incorrect starting point | Reagents allows to come to 20~30℃ before performing assay |
| Too much signal – whole plate turned uniformly blue | Insufficient washing – unbound AV-HRP remaining | Increase number of washes carefully |
| | Too much AV-HRP | Check dilution |
| | Plate sealer or reservoir reused, resulting in presence of residual AV-HRP | Use fresh plate sealer and reagent reservoir for each step |

| Problem | Possible Cause | Solution |
| --- | --- | --- |
| Standard curve achieved but poor discrimination between point | Plate not developed long enough | Increase substrate solution incubation time |
| | Improper calculation of standard curve dilution | Check dilution, make new standard curve |
| No signal when a signal is expected, but standard curve looks fine | Sample matrix is masking detection | More diluted sample recommended |
| Samples are reading too high, but standard curve is fine | Samples contain protein levels above assay range | Dilute samples and run again |
| Edge effect | Uneven temperature around work surface | Avoid incubating plate in areas where environmental conditions vary.<br>Use plate sealer. |

## REFERENCES

1. Castellino F.J. and Ploplis V.A., 2005, Thromb Haemost. 93:647-654
2. Wun T.C., 1988, Crit Rev Biotechnol. 8:131-148

# Product Manual

**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

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