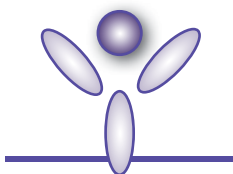




Enabling Legendary Discovery™

# LEGEND MAX™

ELISA Kit



## Human PLAUR (uPAR)

Cat. No. 448407

ELISA Kit for Accurate Quantitation of  
PLAUR (uPAR) from Cell Culture Supernatant, Serum,  
Plasma, Urine, and Other Biological Fluids

BioLegend, Inc.  
[biolegend.com](http://biolegend.com)



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***It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.***

*For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of BioLegend is strictly prohibited.*

**Introduction:**

PLAUR (uPAR), also known as CD87, is a urokinase-type plasminogen activator system consisting of proteinase, receptor, and inhibitor. PLAUR is involved in proteolysis, cell migration and tissue remodeling by proteolysis, signal transduction and chemokine-like activities. It predominately plays roles in cell activation, adhesion, migration, and extravasation in blood cells.

High serum levels of soluble PLAUR (uPAR) are associated with lower survival rates in cancer and HIV-1 patients. Research on COVID-19 suggests that levels of PLAUR (uPAR) are increased in COVID-19 patients that are hospitalized and are in critical condition.

BioLegend’s LEGEND MAX™ PLAUR (uPAR) ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate. The plate is pre-coated with a mouse monoclonal capture antibody. The detection antibody is a biotinylated mouse monoclonal antibody. This kit is specifically designed for the accurate quantitation of human PLAUR (uPAR) from cell culture supernatant, serum, plasma, urine, and other biological fluids. It is analytically validated with ready-to-use reagents.

**Materials Provided:**

| Description                                      | Quantity | Volume      | Part #    |
|--|----------|-------------|-----------|
| PLAUR (uPAR) Pre-Coated 96-well Strip Microplate | 1 plate  |             | 750002416 |
| PLAUR (uPAR) Detection Antibody                  | 1 bottle | 12 mL       | 750002348 |
| PLAUR (uPAR) Standard                            | 1 vial   | lyophilized | 750002419 |
| Avidin HRP                                       | 1 bottle | 12 mL       | 77897     |
| Assay Buffer B                                   | 1 bottle | 25 mL       | 79128     |
| Wash Buffer (20x)                                | 1 bottle | 50 mL       | 78233     |
| Substrate Solution F                             | 1 bottle | 12 mL       | 79132     |
| Stop Solution                                    | 1 bottle | 12 mL       | 79133     |
| Plate Sealers                                    | 4 sheets |             | 78101     |

**Materials to be Provided by the End-User:**

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Polypropylene tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

**Storage Information:**

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

| Opened or Reconstituted Components |   |
|------------------------------------|---|
| Microplate wells                   | If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month. |
| Standard                           | The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.   |
| Detection Antibody                 | Store opened reagents between 2°C and 8°C and use within one month.   |
| Avidin-HRP                         |   |
| Assay Buffer B                     |   |
| Wash Buffer (20X)                  |   |
| Substrate Solution F               |   |
| Stop Solution                      |   |

**Health Hazard Warnings:**

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend’s website for details ([www.biolegend.com/msds](http://www.biolegend.com/msds)).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.

- To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
- Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
- Before disposing of the plate, rinse it with an excess amount of tap water.

**Specimen Collection and Handling:**

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x g. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Urine: Collect first-morning, mid-stream urine samples in a sterile container. To remove heavy sediment, centrifuge for 10 minutes at 1,000 x g. Remove supernatant and assay immediately or store at < -70°C. Avoid repeated freeze-thaw cycles.

**Reagent and Sample Preparation:**

Note: All reagents should be diluted immediately prior to use.

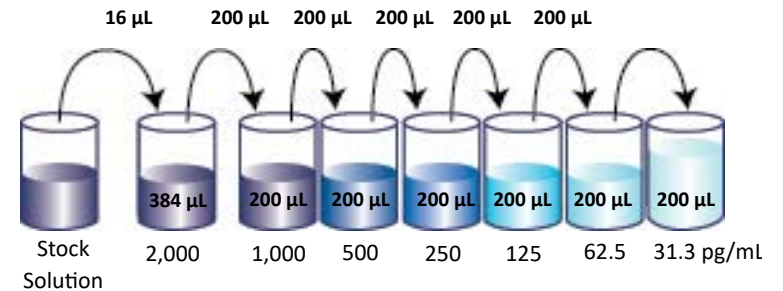
- Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
- Reconstitute the lyophilized Human PLAUR (uPAR) Standard by adding the volume of Assay Buffer B to make the 50,000 pg/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.

- In general, 20-fold dilution in Assay Buffer B is recommended for healthy serum and plasma samples and a 10-fold dilution is recommended for healthy urine samples. Samples can be diluted in Assay Buffer B to fit within the range of the assay as determined by the end user.

**Assay Procedure:**

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

- Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
- If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
- Prepare 400 µL of the 2,000 pg/mL top standard by adding 16 µL of the 50,000 pg/mL of the standard stock solution in 384 µL of Assay Buffer B. Perform six two-fold serial dilutions of the 2,000 pg/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the Human PLAUR (uPAR) standard concentrations in the tubes are 2,000 pg/mL, 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, and 62.5 pg/mL, respectively. Assay Buffer B serves as the zero standard (0 pg/mL).



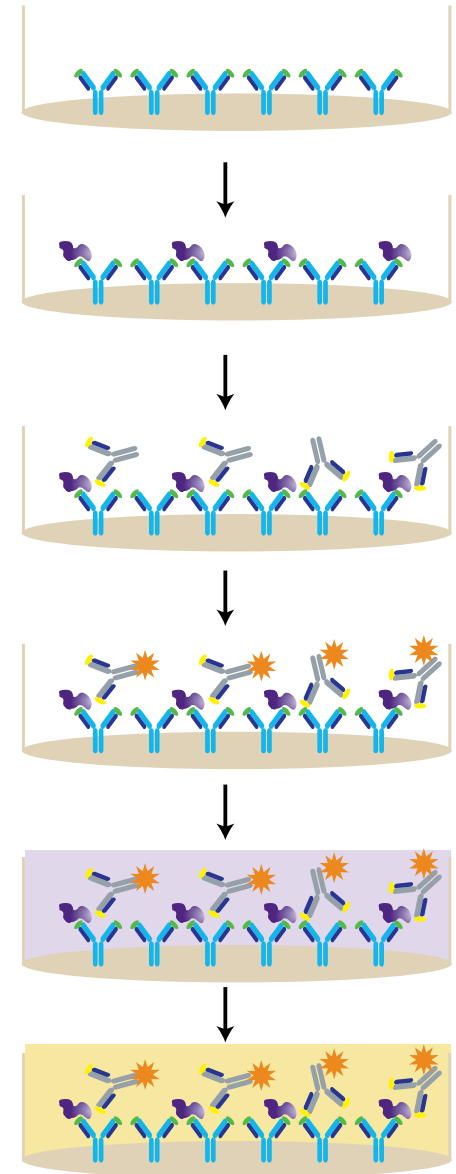
- Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well ; blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.
- Add 50 µL of Assay Buffer B to each well that will contain either standard dilutions or samples. Then add 50 µL of standard dilutions or samples to the appropriate wells.
- Seal the plate with a Plate Sealer included in the kit and incubate the plate for 2 hours at room temperature with shaking.
- Discard the contents of the plate into a sink, then wash the plate 4 times

with 1X Wash Buffer as in step 4.

8. Add 100  $\mu$ L of Human PLAUR (uPAR) Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.
9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
10. Add 100  $\mu$ L of Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
11. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
12. Add 100  $\mu$ L of Substrate Solution F to each well and incubate for 10 minutes in the dark. Wells containing Human PLAUR (uPAR) should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
13. Stop the reaction by adding 100  $\mu$ L of Stop Solution to each well. The solution color should change from blue to yellow.
14. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

### Assay Procedure Summary

1. Wash 4 times.  
Add 50  $\mu$ L Assay Buffer B to standard wells and sample wells
2. Add 50  $\mu$ L of standard or sample, incubate 2 hr, RT, shaking
3. Wash 4 times  
Add 100  $\mu$ L of Detection Antibody solution  
Incubate 1 hr, RT, shaking
4. Wash 4 times  
Add 100  $\mu$ L Avidin-HRP solution  
Incubate 30 min, RT, shaking
5. Wash 5 times  
Add 100  $\mu$ L Substrate Solution F  
Incubate 10 min, RT, in the dark
6. Add 100  $\mu$ L Stop Solution
7. Read absorbance at 450 nm and 570 nm



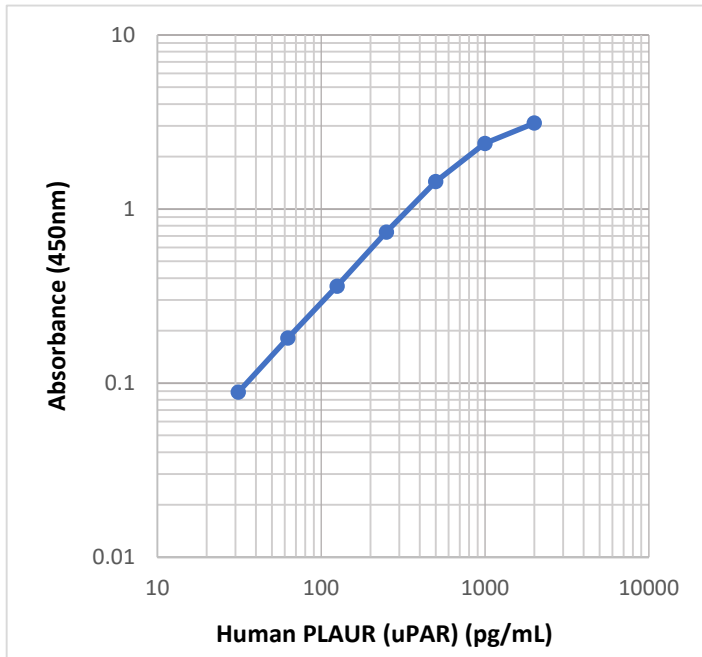
**Calculation of Results:**

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the cytokine concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

**Typical Data:**

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



**Performance Characteristics:**

**Specificity:** This kit recognizes natural and recombinant Human PLAUR (uPAR). No cross reactivity was observed when this kit was used to analyze the following recombinant proteins at 20ng/mL:

|       |  |
|-------|--|
| Human | uPA, IL-2, IL-1b, IL-12, IL-13, CNTF, RANTES/CCL5, TGF- $\alpha$ , CCL2 (MCP1), PDGF-BB, TNF- $\alpha$ , IL-1 $\alpha$ , CCL3(MIP-1 $\alpha$ ) |
|-------|--|

No interference was observed when this kit was used with uPA at concentrations of up to 20ng/mL.

**Sensitivity:** The average minimum detectable concentration of Human PLAUR (uPAR) is 6.15 pg/mL (n=6). The range of calculated minimum detectable concentrations was 5.07 - 6.86 pg/mL.

**Recovery:** Recombinant or natural Human PLAUR (uPAR) at 3 different concentrations was spiked into different human samples of Serum, Citrate Plasma, EDTA Plasma, and Heparin Plasma. Sample recovery was then analyzed with the LEGEND MAX™ Human PLAUR (uPAR) ELISA Kit.

| Sample Type              | N | % Recovery |
|--------------------------|---|------------|
| Serum                    | 6 | 114.1      |
| Citrate Plasma           | 6 | 113.0      |
| EDTA Plasma              | 6 | 114.3      |
| Heparin Plasma           | 4 | 93.3       |
| Urine                    | 3 | 89.2       |
| Cell Culture Supernatant | 3 | 101.5      |

**Linearity:** Human samples, spiked with or containing high concentrations of human PLAUR (uPAR), were diluted 2 fold in serial in Assay Buffer B to produce samples within the dynamic range of the kit. Samples were then assayed to determine the dilutional linearity.

| Sample Type              | N | % Recovery |
|--------------------------|---|------------|
| Serum                    | 6 | 110.1      |
| Citrate Plasma           | 6 | 110.6      |
| EDTA Plasma              | 6 | 105.4      |
| Heparin Plasma           | 6 | 107.1      |
| Urine                    | 3 | 91.8       |
| Cell Culture Supernatant | 4 | 100.5      |

**Intra-Assay Precision:** Two samples containing different Human PLAUR (uPAR) concentrations were tested on one plate with 10 replicates.

| Concentration              | Sample 1 | Sample 2 |
|----------------------------|----------|----------|
| Number of Replicates       | 10       | 10       |
| Mean Concentration (pg/mL) | 215.9    | 45.56    |
| Standard Deviation         | 17.13    | 2.78     |
| %CV                        | 7.93     | 6.10     |

**Inter-Assay Precision:** Two samples containing different PLAUR (uPAR) concentrations were tested in ten independent assays.

| Concentration              | Sample 1 | Sample 2 |
|----------------------------|----------|----------|
| Number of Assays           | 10       | 10       |
| Mean Concentration (pg/mL) | 205.37   | 42.97    |
| Standard Deviation         | 13.29    | 4.07     |
| %CV                        | 6.47     | 9.47     |

**Biological Samples:** PBMCs (peripheral blood mononuclear cells) were stimulated with PHA (3 ug/mL) for 3 days and harvested along with control, unstimulated PBMCs. Cell culture supernatant was assayed for natural human PLAUR (uPAR).

|                       | Unstimulated PBMC Cell Culture Supernatant | PHA-stimulated PBMC Cell Culture Supernatant |
|-----------------------|--|--|
| Concentration (pg/mL) | 1162.0155                                  | 4926.264                                     |

Healthy serum, plasma, and urine samples were assayed at the recommended dilution factors for natural human PLAUR (uPAR).

| Sample Type    | N | Concentration (pg/mL) |
|----------------|---|-----------------------|
| Serum          | 4 | 2272.36               |
| Citrate Plasma | 4 | 1865.82               |
| EDTA Plasma    | 4 | 1832.12               |
| Heparin Plasma | 4 | 1820.51               |

|       |   |         |
|-------|---|---------|
| Urine | 2 | 1004.08 |
|-------|---|---------|

**Troubleshooting Guide:**

| Problem                           | Probable Cause   | Solution  |
|-----------------------------------|--|---|
| High Background                   | Background wells were contaminated   | Avoid cross-well contamination by using the provided plate sealers.<br>Use multichannel pipettes and change tips between pipetting samples and reagents.        |
|                                   | Insufficient washes  | Increase number of washes.<br>Increase soaking time between washes prior to addition of substrate solution.   |
|                                   | TMB Substrate Solution was contaminated  | TMB Substrate Solution should be clear and colorless prior to addition to wells.<br>Use a clean container prior to pipetting substrate solution into wells.     |
| No or poor signal                 | Detection Antibody, Avidin-HRP or Substrate solution were NOT added                | Rerun the assay and follow the protocol.  |
|                                   | Wrong reagent or reagents were added in wrong sequential order                     |   |
|                                   | Insufficient plate agitation   | The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing. |
|                                   | The wash buffer contains Sodium Azide (NaN <sub>3</sub> )                          | Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.  |
| Low or poor standard curve signal | Incubations were done at an inappropriate temperature, timing or without agitation | Rerun the assay and follow the protocol.  |
|                                   | The standard was incorrectly reconstituted or diluted                              | Adjust the calculations and follow the protocol.  |
|                                   | Standard was inappropriately stored  | Store the reconstituted standard stock solution in polypropylene vials at -70°C.<br>Avoid repeated freeze-thaw cycles.  |
|                                   | Reagents added to wells with incorrect concentrations                              | Check for pipetting errors and the correct reagent volume.  |

| Problem   | Probable Cause  | Solution  |
|---|---|---|
| Signal is high, standard curves have saturated signal | Standard reconstituted with less volume than required   | Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.  |
|   | Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long | Rerun the assay and follow the protocol.  |
| Sample readings are out of range                      | Samples contain no or below detectable levels of the analyte  | If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications. |
|   | Samples contain analyte concentrations greater than highest standard point                          | Samples may require dilution and analysis.  |
| High variation in samples and/or standards            | Multichannel pipette errors   | Confirm that pipette calibrations are accurate.   |
|   | Plate washing was not adequate or uniform   | Ensure pipette tips are tightly secured.<br>Ensure uniformity in all wash steps.  |
|   | Non-homogenous samples  | Thoroughly mix samples before assaying.   |
|   | Samples may have high particulate matter  | Remove particulate matter by centrifugation.  |
|   | Cross-well contamination  | Do not reuse plate sealers.<br><br>Always change tips for reagent additions.<br>Ensure that pipette tips do not touch the reagents on the plate.            |

|                             |    |   |   |   |   |   |   |   |  |
|-----------------------------|----|---|---|---|---|---|---|---|--|
| <b>ELISA Plate Template</b> | 12 |   |   |   |   |   |   |   |  |
|                             | 11 |   |   |   |   |   |   |   |  |
|                             | 10 |   |   |   |   |   |   |   |  |
|                             | 9  |   |   |   |   |   |   |   |  |
|                             | 8  |   |   |   |   |   |   |   |  |
|                             | 7  |   |   |   |   |   |   |   |  |
|                             | 6  |   |   |   |   |   |   |   |  |
|                             | 5  |   |   |   |   |   |   |   |  |
|                             | 4  |   |   |   |   |   |   |   |  |
|                             | 3  |   |   |   |   |   |   |   |  |
|                             | 2  |   |   |   |   |   |   |   |  |
|                             | 1  |   |   |   |   |   |   |   |  |
|                             | A  | B | C | D | E | F | G | H |  |





**Legend Max™ Kits are manufactured by BioLegend Inc.  
8999 BioLegend Way  
San Diego, CA 92121  
Tel: 1.858.768.5800  
Tel: US & Canada Toll-Free: 1.877.Bio.Legend (1.877.246.5343)  
Fax: 1.877.455.9587  
Email: [info@biolegend.com](mailto:info@biolegend.com)  
[Biolegend.com](http://Biolegend.com)**

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please visit our website at: [biolegend.com](http://biolegend.com)