

APPLICATION GUIDE

Human PSP/REG1A ELISA Kit

Catalog No. EA200007

For quantitative detection of human PSP/REG1A in serum, plasma and other biological fluids

**FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURE**

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Principle of the Assay

Pancreatic stone protein (PSP), also known as lithostathine, and regenerating protein (REG1A). It is a secreted, type I subclass member of the regenerating protein family, and has been found both in blood and pancreatic secretions. Mature human PSP/REG1A shares 76% and 69% aa sequence identity with mouse and rat PSP/REG1A, respectively. PSP/REG1A is constitutively expressed by pancreatic acinar cells, and is also induced in proliferating cell types such as colonic epithelium, islet beta-cells, and multiple tumor types. The 16 kDa precursor of PSP/REG1A has been observed mainly in the pancreas and is transiently elevated in acute and chronic pancreatitis. PSP/REG1A plays a role in blocking apoptosis, and is upregulated by both IL-22 and IL-6. Under healthy conditions PSP/REG1A is expressed at low levels in the pancreas. Upon focal or systemic inflammation, PSP/REG1A is strongly increased. PSP/REG1A is elevated in various conditions such as sepsis, ventilator associated pneumonia (VAP), chronic obstructive pulmonary disease (COPD) exacerbation, and type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). These observations indicated that PSP/REG1A might respond to infectious conditions and organ failure. The use of the PSP/REG1A as a biomarker has been confirmed the high diagnostic accuracy for sepsis. In addition, evidences obtained regarding PSP/REG1A and its isoforms on regeneration, growth and mitogenesis in gastrointestinal neoplastic diseases suggest that PSP/REG1A can be used as biomarker in the prediction of prognosis for neoplastic diseases.

This sandwich ELISA is used to measure human PSP/REG1A in serum, plasma and other biological fluid samples. Microtitration wells coated with anti-human PSP/REG1A capture antibody are exposed to test specimens. The PSP/REG1A antigen in the specimen is specifically captured onto the immobilized antibody during specimen incubation. The captured PSP/REG1A antigen is then reacted with a biotinylated human PSP/REG1A detection antibody. Subsequently, Streptavidin-HRP conjugate is then added. After wash, specifically bound enzyme conjugate is detected by reaction with the Substrate Solution, tetramethylbenzidine (TMB). The assay is measured spectrophotometrically to indicate the level of PSP/REG1A present in a sample.

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Kit Presentation

Materials Supplied

The reagents supplied in this pack are for Research Use Only.

Description	Quantity
PSP/REG1A Antibody Coated 96-well Plate in foil pouch with desiccant	1
Recombinant human PSP/REG1A Standard (50ng/mL)	0.1 mL
Biotinylated PSP/REG1A Detection Antibody (100x)	120 µL
Streptavidin Conjugated Horseradish Peroxidase (100x)	120 µL
Assay Buffer	30 mL
Sample Diluent	40 mL
Substrate Solution (TMB)	12 mL
Stop Solution (1N HCl)	12 mL
Wash Buffer (20x)	60 mL
Plate Sealer	3

Additional Requirements for Manual Processing

1. Horizontal orbital plate shaker capable of maintaining a speed of 450±50 rpm.
2. Disposable tip micropipettes to deliver volumes of 5µL, 10 µL, 25 µL, 100 µL and 200 µL (multichannel pipette preferred for dispensing reagents into microtiter plates).
3. Distilled or deionized water.
4. Clean, disposable plastic/glass test tubes, approximate capacities 5mL and 10mL.
5. Range of standard, clean volumetric laboratory glassware consisting of, at least, 15 mL and 100 mL beakers, 1 L graduated cylinder, 1 mL, 5 mL, and 10 mL pipettes.
6. Absorbent paper towels.
7. Automatic microplate washer or laboratory wash bottle.
8. Microplate reader with 450nm filter.
9. Latex gloves, safety glasses and other appropriate protective garments.
10. Biohazard waste containers.
11. Safety pipetting devices for 1 mL or larger pipettes.
12. Timer.

Storage and Stability

All reagents should be stored at 2-8°C and should not be used beyond the expiration date on the label. Once opened, microtitration strips may be stored at 2-8°C until the expiration date on the label, provided that desiccated conditions are maintained. Unused strips should be returned to their original foil pouch along with the sachet of desiccant. Secure open foil pouch using zip top before storage. The working strength Wash Buffer should

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not be stored for longer than 3 weeks at 2-8°C. It is recommended that Wash buffer be freshly diluted before each assay. If the working strength buffer becomes visibly cloudy or develops precipitate during the 3 weeks, do not use it.

Indications of Deterioration

The human PSP/REG1A Assay kit may be considered to have deteriorated if:

1. Reagents becoming visibly cloudy or develop precipitate. Note: Concentrated Wash buffer, when cold, normally develops crystalline precipitates, which re-dissolve on heating at 37°C.
2. The Substrate Solution turns dark blue. This is likely to be caused by chemical contamination of the Substrate Solution.

Warnings and Precaution

Safety

1. The reagents supplied in this kit are for **Research use only**.
2. Caution: All blood products should be treated as potentially infectious.

Essential precautions can be summarized as follows:

- >do not pipette by mouth.
 - >Wear disposable gloves during all specimen and assay manipulations.
 - >Avoid use of sharp or pointed liquid handling devices, which may puncture skin.
 - >Do not smoke, eat or drink in the laboratory work area.
 - >Avoid splashing of liquid specimens and reagents and the formation of aerosols.
 - >Wash hands thoroughly on completion of a manipulation.
 - >The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at Biosafety Level 2.
3. The kit contains reagent systems which are optimized and balanced for each kit lot. Do not interchange reagents from kits with different lot numbers. Do not interchange vial caps or stoppers either within or between kits.
 4. The Substrate Solution and Stop Solution in this kit contain ingredients that can irritate the skin and cause eye damage. Handle them with care and wear suitable protective clothing and eye/face protection. In case of contact with skin or eyes, immediately flush the affected area with plenty of water. For eyes, obtain medical attention.
 5. Disposal or decontamination of fluid in the waste reservoir from either the plate washer or trap for vacuum line in the manual system should be in accordance with guidelines set forth in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910,1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.

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Technical Suggestions

1. This kit should be used in strict accordance with the instructions in the Package Insert.
2. Do not use the kit after the expiration date printed on the outer carton label.
3. Do not cross contaminate reagents.
4. To ensure accurate results and avoid cross-contamination, use proper adhesive plate sealers during incubation steps, and change pipette tips when adding each standard and sample. Multi-channel pipettes are recommended for large assays. Always use fresh pipette tips when drawing from stock reagent bottles.
5. All reagents should be added to the plate in the same order.
6. Protect Substrate Solution from light.
7. If the Stop Solution does not mix thoroughly with the Substrate Solution, the color in the wells may appear green after adding stop solution. Gently tap the plate or pipette up and down to mix until the color in the wells change to yellow (avoid bubbles during this step).
8. Always use clean, preferably disposable, glassware for all reagent preparation.
9. Allow foil bags to warm to room temperature before opening. This avoids condensation on the inner surface of the bag, which may contribute to a deterioration of coated strips intended for future use.
10. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. Follow manufacturer's recommendations for automatic processors.
11. Always keep the upper surface of the microtitration strips free from excess fluid droplets. Reagents and buffer over-spill should be blotted dry on completion of the manipulation.
12. Do not allow the wells to completely dry during an assay.
13. Automatic or semi-automatic EIA processors or liquid handling systems should be qualified specifically for use with the assay by demonstration of equivalence to the manual processing methods.
14. Consistent with good laboratory practice, it is recommended that all pipetting devices (manual or automatic), timers and thermometers are regularly calibrated according to the manufacturer's instructions.
15. Care must be taken to ensure that specimens are dispensed correctly to each test well. If a specimen is inadvertently not added to a well, the result for that well will be non-reactive, regardless of the actual result of the specimen.

Method of Use

Specimen Collection and Storage

The Human PSP/REG1A ELISA is intended for use with serum, plasma and other biological fluid samples. The specimen should be tested as soon as possible. However, if the specimen needs storage, the specimens should be stored frozen at -20°C or below. Do not use self-defrosting freezers. Specimens that have been frozen and thawed should be thoroughly mixed before testing.

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Cell Culture Supernatant - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Rinse Cycle

Efficient rinsing to remove un-complexed components is a fundamental requirement of enzyme immunoassay procedures. Automatic plate washers may be used provided they meet the following criteria: 1. All wells are completely aspirated. 2. All wells are filled to the rim (300 μ L) during the rinse cycle. 3. Wash buffer is dispensed at a good flow rate. 4. The microtitration plate washer must be well maintained to prevent contamination from previous use. Manufacturer's cleaning procedures must be followed diligently

For the rinse cycle, the machine should be set to three washes for the first two rinse cycles and five washes for final rinse cycle. On completion of the cycle, invert the microtitration plate and tap firmly on absorbent paper towels. Check for any residual Wash buffer in the wells and blot dry the upper surface of the wells with a paper towel.

Alternatively, the following manual system may be employed:

1. Discard or aspirate well contents using a vacuum line fitted with a trap.
2. Fill all wells to the brim with wash buffer dispensed from a squeeze-type laboratory wash bottle.
3. Discard or aspirate fluids.
4. Repeat steps 2 and 3, two times (Repeat steps 2 and 3 four times in final wash cycle).
5. Invert the microtitration plate and tap firmly on absorbent paper towels.

Preparation for the Assay

1. *Standard preparation:* Prepare protein standard by diluting 10 μ L of standard stock into 490 μ L (1:50 dilution) of assay buffer. This will give a final concentration of 1000 pg/mL as shown in Table 1.

2. *Sample preparation:* PSP/REG4 concentration must be estimated prior to performing the full experiment by testing a serially diluted representative sample. Select an optimal dilution level such that the final target protein concentration falls near the middle of the assay linear dynamic range. Or refer to published literature for expected concentrations and derive the optimal dilution level based on the expected dynamic range of the kit.

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Proper dilution using sample diluent is needed for samples with PSP/REG1A concentration beyond the highest detection range.

3. *Detection antibody preparation:* Dilute the concentrated biotin conjugated detection antibody 1:100 using assay buffer.
4. *SA-HRP preparation:* Dilute the concentrated streptavidin HRP conjugate 1:100 using assay buffer.
5. *Wash buffer:* Prepare working-strength Wash buffer by diluting 1 part concentrate with 19 parts of distilled or de-ionized water. If a kit is likely to be utilized over a period in excess of 4 weeks, then it is recommended that only enough stock concentrate be diluted sufficient for immediate needs.

Quantitative Assay Procedure

To test quantitatively, a standard curve should be prepared using assay diluent as shown in the table below. Each standard should be run in duplicate.

Table 1: Human PSP/REG1A Quantitative Standard Curve Generation

Standard Number	Concentration of PSP/REG1A (pg/mL)	PSP/REG1A Standard (µL)	Assay Buffer (µL)
1	1000	10	490
2	500	250 of #1	250
3	250	250 of #2	250
4	125	250 of #3	250
5	62.5	250 of #4	250
6	31.25	250 of #5	250
7	15.625	250 of #6	250
8	0		250

Assay Procedure

1. Allow all reagents to reach room temperature (18-25°C).
2. Select sufficient microtitration well strips to accommodate all test specimens, controls and reagent blank. Fit the strips into the holding frame. Label wells according to specimen identity using the letter/number cross-reference system molded into the plastic frame.
3. Dispense 100 µL of each standard and sample into appropriate wells. Note: All standards and samples should be tested in duplicate. Note: Depending on the PSP/REG1A concentration of your sample, dilution using sample diluent may be needed. If the sample PSP/REG1A concentration is not known, you can titrate the original sample.

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4. Incubate for 2 hours at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.
5. Wash the microtitration plate 3 times as described in the Rinse Cycle section.
6. Pipette 100 µL of working concentration detection antibody into each well and incubate for 1 hour at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.
7. Wash the microtitration plate 3 times as described in the Rinse Cycle section.
8. Pipette 100 µL of working concentration Streptavidin HRP conjugate into each well and incubate for 25 minutes at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.
11. Wash the microtitration plate 5 times as described in the Rinse Cycle section.
12. Dispense 100 µL Substrate Solution into each well. A multichannel pipette should be used for best results. Leave at room temperature (18-25°C) and protected from direct sunlight for 20-25 minutes.
13. Stop the reaction by adding 100 µL of Stop Solution to each well. The blue solution should change to a uniform yellow color. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.
14. Immediately after adding the Stop solution, read the absorbance values at 450 nm using a microtitration plate reader.

Interpretation of Results

Quantitative Analysis

Average the duplicate readings for each standard and sample, and subtract the average zero standard optical density (O.D.).

A 4-parameter logistic (4-PL) or a linear regression model providing a point-to-point curve fitting provides acceptable results. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) or a linear regression curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Do not force the line to be linear. The concentration of the samples can be found directly from the standard curve.

Table 2. Example Data at 450nm.

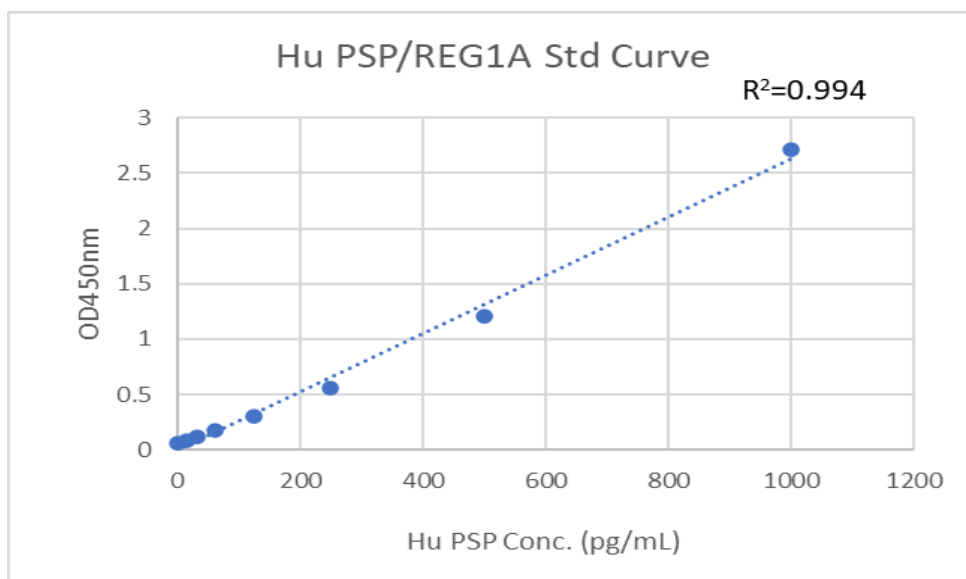
Standards	450 nm absorbance
Standard 1 (1000 pg/mL)	2.71125
Standard 2 (500 pg/mL)	1.20355

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Standard 3 (250 pg/mL)	0.55615
Standard 4 (125 pg/mL)	0.2985
Standard 5 (62.5 pg/mL)	0.17085
Standard 6 (31.25 pg/mL)	0.11615
Standard 7 (15.625 pg/mL)	0.0856
Standard 8 (0 pg/mL)	0.062

Typical Human PSP/REG1A ELISA Kit Standard Curve

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



Note: This standard curve is only an example and should not be used to generate any results.

Performance Characteristics

1. Recovery

The recovery of human PSP/REG1A spiked to three different-levels of the assay range in diluted samples was evaluated

Sample Type	Average % Recovery
Serum	100.3%
EDTA-plasma	94.9%
Heparin-plasma	96.4%
Cell culture media	96.7%

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2. Linearity

To assess the linearity of the assay, neat or human PSP/REG1A spiked samples were diluted to produce samples with values within the dynamic range of the assay.

		Cell culture media	Heparin-plasma	EDTA-plasma	Serum
1:2	%Expected	96.66	99.64	104.58	100.15
1:4	%Expected	96.36	108.71	110.68	106.24
1:8	%Expected	104.89	106.30	112.80	111.06

3. Sensitivity: 5.27pg/mL

4. Precision

Human serum, plasma and culture media samples with different levels of PSP/REG1A were assayed 10 times each on three different assays. The intra-assay CV percentage and inter-assay CV percentage were calculated.

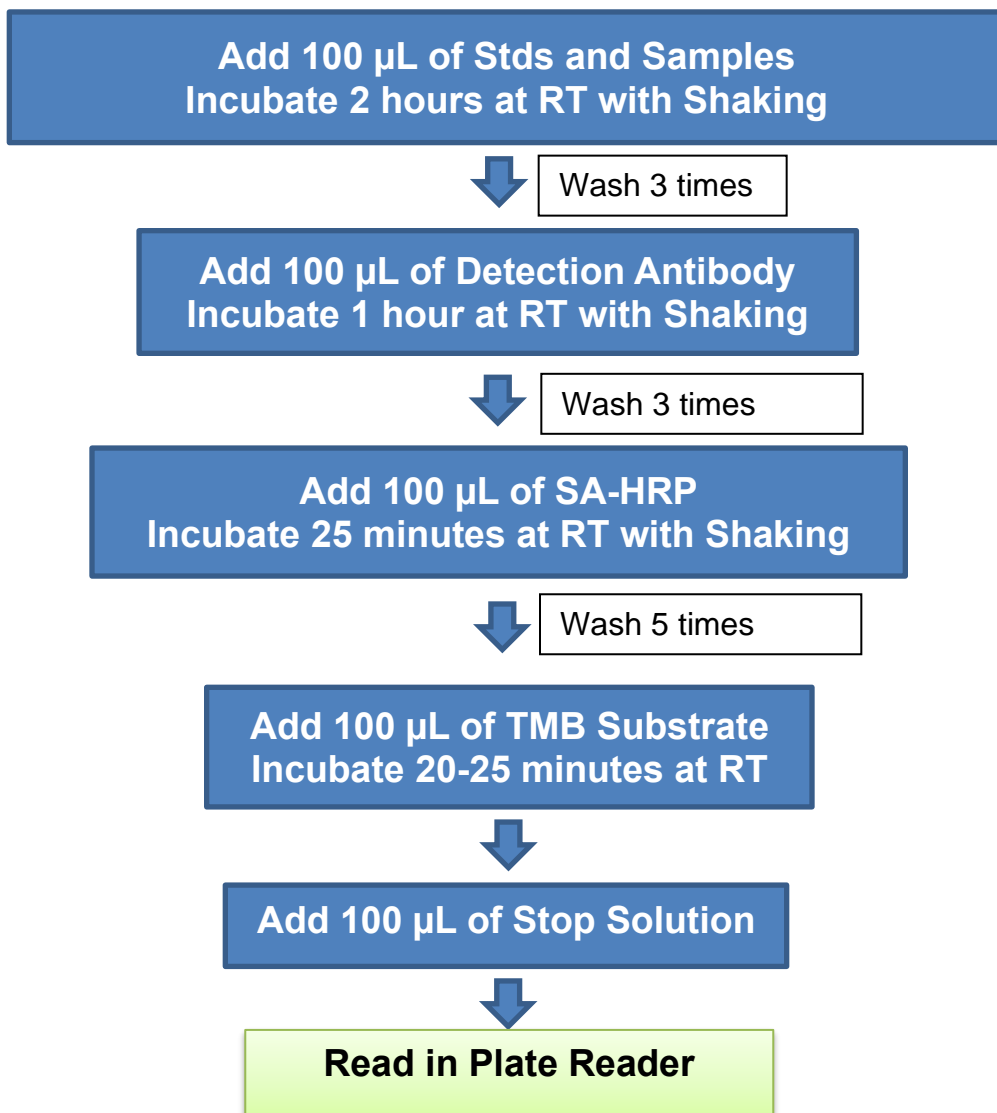
Sample	%CV in Assay 1	%CV in Assay 2	%CV in Assay 3	Ave %CV
Serum (n=10)	4.93	3.72	3.17	3.94
EDTA-Plasma (n=10)	4.09	2.58	4.81	3.83
Heparin-Plasma (n=10)	2.44	2.26	3.47	2.72
Culture Media (n=10)	4.36	2.26	3.08	3.23

Sample	Mean (pg/ml) in assay1	Mean (pg/ml) in assay2	Mean (pg/ml) in assay3	Ave (pg/ml)	SD	%CV
Serum (n=10)	519.37	461.04	488.45	489.62	29.18	5.96
EDTA-Plasma (n=10)	260.41	268.52	280.55	269.83	10.13	3.76
Heparin-Plasma (n=10)	304.07	318.64	341.26	321.32	18.74	5.83
Culture Media (n=10)	168.01	170.61	169.22	169.28	1.30	0.77

Limitations of Use

1. Assay values determined using assays from different manufacturers or different methods may not be used interchangeably.
2. The assay cannot be used to quantitate samples with PSP/REG1A assay values greater than the highest standard without further serial dilution of the samples.

Assay Flowchart





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Contact Information:

OriGene Technologies, Inc
9620 Medical Center Drive
Rockville, MD 20850
Tel: 888.267.4436
Fax: 301.340.9254
Web: www.origene.com

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