OriGene Technologies Inc.
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Human PSG1 ELISA Kit

Catalog Number:EA102675

Assay Principle

The OriGene Human PSG1 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human PSG1 with a 96-well strip plate that is pre-coated with antibody specific for PSG1. The detection antibody is a biotinylated antibody specific for PSG1. The capture antibody is A monoclonal antibody from mouse, the detection antibody is a biotinylated detection polyclonal antibody from goat. The kit contains recombinant Human PSG1 with immunogen: Expression system for standard: NSO, Immunogen sequence: Q35-P419. The kit is analytically validated with ready to use reagents.

To measure Human PSG1, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Human PSG1 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human PSG1 in the sample.

Overview

Product Name	Human PSG1 ELISA Kit
Reactive Species	Human
Size	96wells/kit, with removable strips.
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of human PSG1. 96wells/kit, with removable strips.
Sensitivity	<50pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	1.56ng/ml-100ng/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)
Uniprot ID	P11464

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Technical Details

Capture/Detection Antibodies	Thecaptureantibodyis Amonoclonalantibodyfrommouse,thedetectionantibodyisa biotinylateddetection polyclonalantibodyfromgoat.
Specificity	NaturalandrecombinantHumanPSG1
Immunogen	Expression system for standard: NSO, Immunogen sequence: Q35-P419
Cross Reactivity	There is no detectable cross-reactivity with other relevant proteins.

Notice Before Application

Please read the following instructions before starting the experiment.

1. Toinspectthevalidityofexperimentoperationandtheappropriatenessofsampledilutionproportion,pilotexperimentusing standardsandasmall numberofsamplesisrecommended.
2. BeforeusingtheKit,spintubesandbringdownallcomponentstothebottomoftubes.
3. Don’tlet96-wellplatedry,fordryplatewillinactivateactivecomponentsonplate.
4. Don’treusetipsandtubestoavoidcrosscontamination.
5. Avoidusingthereagentsfromdifferentbatchestogether.

Kit Components / Materials Provided

Description	***Quantity***	***Volume***
Anti-Human PSG1 Pre-coated 96-well strip microplate	***1***	***12 strips of 8 wells***
Human PSG1 Standard	***2***	***100ng/tube***
Human PSG1 Biotinylated antibody (100x)	***1***	***130 µl***
Avidin-Biotin-Peroxidase Complex (100x)	***1***	***130 µl***
Sample Diluent	***1***	***30ml***
Antibody Diluent	***1***	***12ml***
Avidin-Biotin-Peroxidase Diluent	***1***	***12ml***
Color Developing Reagent (TMB)	***1***	***10ml***
Stop Solution	***1***	***10ml***
Plate Sealers	***4***	***Piece***

*Whythereisnowashbuffer?OurAvidin-Biotin-PeroxidaseDiluentcontainsthedetergent(TWEEN)normallypresentinothercompanies'ELISA kits.Thissavesyouthestep ofhavingtowashwiththespecialwashbufferandachievesimilarorbettersignaltonoiseratio.Thewashcanuse regularwashbuffers(PBS,TBSetc.)commonlyfoundinlabs.

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Required Materials That Are Not Supplied

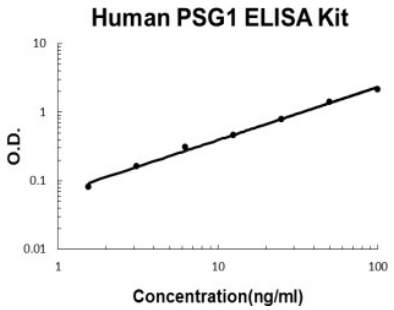
Microplate Reader capable of reading absorbance at 450nm. Automated plate
washer(optional)
1000ml of 1X wash buffer (TBS or PBS)
Pipettes and pipette tips capable of precisely dispensing 0.5µl through 1ml volumes of aqueous solutions. Multichannel pipettes
are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Human PSG1 ELISA Kit (EA102675) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (ng/ml)	***0***	***1.56***	***3.12***	***6.25***	***12.5***	***25***	***50***	***100***
O.D.	***0.013***	***0.082***	***0.164***	***0.313***	***0.466***	***0.796***	***1.403***	***2.156***

Human PSG1 ELISA Kit Standard Curve



A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Intra / Inter Assay Variability

OriGene spend greate ffortsindocumenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision across assays): Three samples of known concentration were tested in separate assays to assess inter-assay

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precision.

	Intra-AssayPrecision			Inter-AssayPrecision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	2327	9714	48035	2915	11475	48927
Standarddeviation	114.02	456.56	1969.44	163.24	585.23	3229.18
CV(%)	4.9%	4.7%	4.1%	5.6%	5.1%	6.6%

Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/ml)	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean(pg/ml)	Standard Deviation	CV(%)
Sample1	2327	2279	2306	2344	2314	24.27	1.0%
Sample2	9714	10138	10569	10990	10352	476.17	4.5%
Sample3	48035	41237	47332	49070	46418	3054.74	6.5%

*number of samples for each test n=16.

Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-25min) is based on 37°C.
Wash buffer	Prepare 1000ml of 1X PBS or TBS for wash buffer.
Biotinylated Anti-Human PSG1 antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human PSG1 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100µl by adding 1µl of Biotinylated antibody (100x) to 99µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin- Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100µl by adding 1µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Human PSG1 Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the

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	experiment.Useone100ngoflyophilizedHumanPSG1standardforeachexperiment.Gentlyspinthevial priortouse. Reconstitutethestandardtoastockconcentrationof100ng/mlusing1mlofsamplediluent. Allowthestandardtositfora minimumof10minuteswithgentleagitationpriortomakingdilutions.
Microplate	Theincludedmicroplateiscoatedwithcaptureantibodiesandready-to-use.Itdoesnotrequireadditional washingorblocking. Theunusedwellstripsshouldbesealedandstoredintheoriginalpackaging.

Dilution of Human PSG1 Standard

1. Numbertubes1-8.FinalConcentrationstobeTube#1–100000pg/ml,#2–50000pg/ml,#3–25000pg/ml,#4–12500pg/ml,#5–6250pg/ml,#6–3125pg/ml,#7–1562.5pg/ml,#8–0.0(Blank).
2. Forstandard#1,add1000µlofundilutedstandardstocksolutiontotube#1.
3. Add300µlofsamplediluenttotubes#2-7.
4. Togeneratestandard#2,add300µlofstandard#1fromtube#1totube#2forafinalvolumeof600µl.Mixthoroughly.
5. Togeneratestandard#3,add300µlofstandard#2fromtube#2totube#3forafinalvolumeof600µl.Mixthoroughly.
6. Continuetheserialdilutionfortube#4-7.
7. Tube#8isablankstandardtobeusedwitheveryexperiment.

Sample Preparation andStorage

Thesesamplecollectioninstructionsandstorageconditionsareintendedasageneralguidelineandthesamplestabilityhasnotbeenevaluated.

Sample Type	Procedure
Cell culture supernatants	Clearsampleofparticulatesbycentrifugation,assayimmediatelyorstoresamplesat-20°C.
Serum	Useaserumseparatortube(SST)andallowserumtoclotatroomtemperatureforaboutfour hours.Then,centrifugefor 15minatapproximately1,000xg.assayimmediatelyorstoresamples at-20°C.
Plasma	CollectplasmausingheparinorEDTAasananticoagulant.Centrifugefor15minatapproximately 1,000xg.Assay immediatelyorstoresamplesat-20°C. *Note:itisimportanttonotuseanticoagulantsotherthantheonesdescribedabovetotreat plasmaforother anticoagulantscouldblocktheantibodybindingsite.
Urine	Collectthefirsturineoftheday,micturatedirectlyintoasterilecontainer.Removeimpuritiesby centrifugation,assay immediatelyoraliquotandstoresamplesat-20°C.

Sample Dilution

Thetargetproteinconcentrationshouldbeestimatedandappropriatesampledilutionsshouldbeselectedsuchthatthefinalprotein concentration lies near the middle of the linear dynamic range of the assay.

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Itisrecommendedtoprepare150µlofsampleforeachreplicatetobeassayed.Thesamplesshouldbedilutedwithsamplediluentandmixed gently.

Assay protocol

Itisrecommendedthatallreagentsandmaterialsbeequilibratedto37°C/roomtemperaturepriortotheexperiment(seePreparationBeforeThe Experimentifyouhavemissedthis information).

1. Prepareallreagentsandworkingstandardsasdirectedpreviously.
2. Removeexcessmicroplatestripsfromtheplateframeandsealandstorethemintheoriginalpackaging.
3. Add100µlofthestandard,samples,orcontrolperwell.Add100µlofthesamplediluentbufferintothecontrolwell(Zerowell).Atleasttwo replicatesofeachstandard,sample, orcontrolisrecommended.
4. Coverwiththeplatesealerprovidedandincubatefor120minutesatRT(or90min.at37°C).
5. Removethecoveranddiscardtheliquidinthewellsintoanappropriatewastereceptacle.Inverttheplateonthebenchtopontoapapertowel andtaptheplatetogentlyblotany remainingliquid.Itisrecommendedthatthewellsarenotallowedtocompletelydryatanytime.
6. Add100µloftheprepared1xBiotinylatedAnti-HumanPSG1antibodytoeachwell.
7. Coverwithplatesealerandincubatefor90minutesatRT(or60minutesat37°C).
8. Washtheplate3timeswiththe1xwashbuffer.
   a. Discardtheliquidinthewellsintoanappropriatewastereceptacle.Then,inverttheplateonthebenchtopontoapapertowelandtapthe platetogentlyblotanyremaining liquid.Itisrecommendedthatthewellsarenotallowedtocompletelydryatanytime.
   b. Add300µlofthe1xwashbuffertoeachassaywell.(Forcleanerbackgroundincubatefor60secondsbetweeneachwash).
   c. Repeatstepsa-b2additionaltimes.
9. Add100µloftheprepared1xAvidin-Biotin-PeroxidaseComplexintoeachwell.Coverwiththeplatesealerprovidedandincubatefor40 minutesatRT(or30minutesat 37°C).
10. Washtheplate5timeswiththe1xwashbuffer.
    a. Discardtheliquidinthewellsintoanappropriatewastereceptacle.Then,inverttheplateonthebenchtopontoapapertowelandtapthe platetogentlyblotanyremaining liquid.Itisrecommendedthatthewellsarenotallowedtocompletelydryatanytime.
    b. Add300µlofthe1xwashbuffertoeachassaywell.(Forcleanerbackgroundincubatefor60secondsbetweeneachwash).
    c. Repeatstepsa-b4additionaltimes.
11. Add90µlofColorDevelopingReagenttoeachwell.Coverwiththeplatesealerprovidedandincubateinthedarkfor30minutesatRT(or 15-25minutesat37°C).(Theoptimal incubationtimemustbeempiricallydetermined.Aguidelinetolookforisblueshadingthetopfourstandard wells,whiletheremainingstandardsremainclear.)
12. Add100µlofStopSolutiontoeachwell.Thecolorshouldimmediatelychangetoyellow.
13. Within30minutesofstoppingthereaction,theO.D.absorbanceshouldbereadwithamicroplatereaderat450nm.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

Itisrecommendedthatastandardcurvebecreatedusingcomputersoftwaretogenerateafourparameterlogistic(4-PL)curve-fit.Afree programcapableofgeneratingafour parameterlogistic(4-PL)curve-fitcanbefoundonlineat:www.myassays.com/four-parameter-logistic- curve.assay.

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Alternatively,plotthemeanabsorbanceforeachstandardagainsttheconcentration.Themeasuredconcentrationinthesamplecanbe interpolatedbyusinglinearregression ofeachaveragerelativeODagainstthestandardcurvegeneratedusingcurvefittingsoftware.Thiswill generateanadequatebutlessprecisefitofthedata.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on PSG1

ThePSG1isasemi-automaticsniperrifle， Pregnancy-specificbeta-1glycoprotein1(PSG-1)isalsoknownasSP1,PSbG1,orB1G1,andis designatedCD66f.Itis mappedto19q13.2.Thehumanplacentaisamultihormonalendocrineorganthatproduceshormones,enzymes,and othermoleculesthatsupportfetalsurvivaland development.Pregnancy-specificbeta-1-glycoprotein(PSBG,PSG)isamajorproductofthe syncytiotrophoblast, reaching concentrations of 100 to 290 mg/l at term in the serum of pregnant women. PSG is a member of the immunoglobulin(Ig)superfamily.