ELISA PRODUCT INFORMATION & MANUAL

Human VSIG4 ELISA Kit (Colorimetric)

NBP2-80355

Sample Insert for Reference Only

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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BACKGROUND

VSIG4 (V-set and immunoglobulin domain containing 4), also known as complement receptor of the immunoglobulin superfamily (CRIg) and Z39Ig, is a type I transmembrane glycoprotein. It is a B7 family-related protein and an Ig superfamily member. In contrast to the B7 family members which contain two IgG domains, VSIG4 contains one complete V-type I g domain and a truncated C-type I g domain. VSIG4 is exclusively expressed on tissue resident macrophages and binds to multimers of C3b and iC3b that are covalently attached to particle surfaces. No VSIG4 expression appears to be present in T and B cells. VSIG4 functions as a negative regulator of T cell activation, and may be involved in the maintenance of peripheral T cell tolerance, and is also identified as a potent suppressor of established inflammation. Mouse VSIG4 is synthesized as a 28 amino acid precursor that contains a signal sequence, an V-type I g domain (aa 36-115), one potential N-linked glycosylation site, and a single transmembrane domain. The V-type I g domain of mouse VSIG4 shares 86% and 8% aa sequence identity with the V-type I g domains of rat and human VSIG4, respectively.

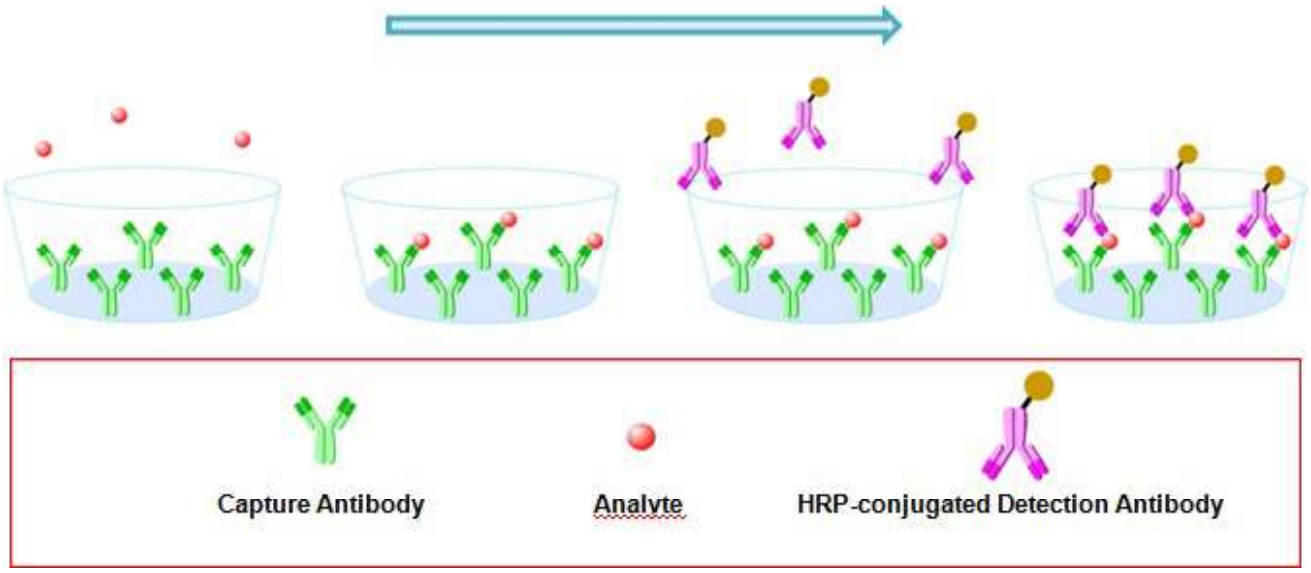
INTENDED USE

For the quantitative determination of Human VSIG4 concentration in cell lysate.

The use of this kit for other sample types need be validated by the end user due to the complexity of natural targets and unpredictable interference.

PRINCIPLE OF THE ASSAY

The principle of this ELISA kit is based on the solid phase sandwich enzyme immunoassay technique. A monoclonal antibody specific for Human VSIG4 has been pre-coated onto well plate strips. Standards and samples are added to the wells and Human VSIG4 present in the sample is bound by the immobilized antibody. After incubation the wells are washed and a horseradish peroxidase conjugated anti-Human VSIG4 antibody is added, producing an antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody a TMB substrate solution is loaded and color develops in proportion to the amount of Human VSIG4 bound. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm (See schematics below).



MATERIALS PROVIDED

Human VSIG4 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with rabbit mAb antibody against Human VSIG4.

Human VSIG4 Detection Antibody - 0.2 mg/mL of rabbit mAb antibody against Human VSIG4 conjugated to horseradish peroxidase (HRP) with preservatives.

Human VSIG4 Standard - Recombinant Human VSIG4 in a buffer with preservatives, lyophilized. The amount of standard is lot specific and indicated on the label of standard vial.

Wash Buffer Concentrate - 25 mL of a 20-fold concentrated solution of buffered surfactant with preservatives.

Dilution Buffer Concentrate - 8 mL of a 20-fold concentrated dilution buffer with preservatives.

Color Reagent A - 13 mL of stabilized hydrogen peroxide.

Color Reagent B - 13 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution - 8 mL of 2 N sulfuric acid.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8℃ and the kit is stable for 6 months upon receipt.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="2">Stored for up to 1 week at 2 - 8℃</td></tr>
<tr><td>Diluted Dilution Buffer</td></tr>
<tr><td>Conjugate</td><td rowspan="3">Stored for up to 1 month at 2 - 8℃</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td><td rowspan="2">After reconstitution, store for up to 1 month at -80℃.
The reconstituted standards should be aliquoted and
avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Microplate Wells</td><td>Return unused strips to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal.
Stored for up to 1 month at 2 - 8℃</td></tr>
</table>

OTHER SUPPLIES REQUIRED

·Microplate reader capable of measuring absorbance at 450 nm

·Pipettes and pipette tips

·Deionized or distilled water

·Multi -channel pipette, squirt bottle, manifold dispenser, or automated microplate washer

·500 mL graduated cylinder

·Tubes for standard dilution

·Well plate cover or seals

PRECAUTIONS

1. This kit is **for research use only** and is not for use in diagnostic or therapeutic procedures.

2. The kit should not be used beyond the expiration date.

3. Do not mix reagents from different lots.

4. The kit is designed and tested to detect the specific targets and samples shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.

SAFETY INSTRUCTIONS

5. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risk.

6. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.

7. Personal protective equipments such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

TECHINICAL TIPS

8. Bring all reagents and samples to room temperature before use.

9. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.

10. A standard curve should be generated for each set of sample assayed. DO NOT USE the standard curves from other plates or other days.

11. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.

12. Read the absorbance of each well within 20 minutes after adding the stop solution.

SAMPLE COLLECTION AND STORAGE

1. Rinse cells two times with PBS and remove any remaining PBS after the second rinse.

2. Incubate cells at 2 x 107 cells/mL in NP-40 Lysis Buffer for 30 minutes on ice.

3. Centrifuge samples at 12000 x g for 10 minutes and transfer the supernatant to a clean test tube.

4. Measure total protein concentration of samples by routine quantitation methods like BCA.

5. Aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Beyotime NP-40 Lysis Buffer (Catalog P0013F) is suggested. (Please add 0.5%PMSF before use)

Note:

The sample should be diluted to within the working range of the assay in 1× dilution buffer. The exact dilution must be determined based on the concentration of specific target in individual samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.

Wash Buffer - Prepare 1× wash buffer by adding 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 400 mL of Wash Buffer.

Dilution Buffer - Prepare 1× dilution buffer by adding 5 mL of Dilution Buffer Concentrate to deionized or distilled water to prepare 100 mL of Dilution Buffer.

Detection Antibody - Centrifuge at 10,000 x g for 20 seconds. Dilute to **work concentration** of 0.25 µg/mL in Dilution Buffer before use.

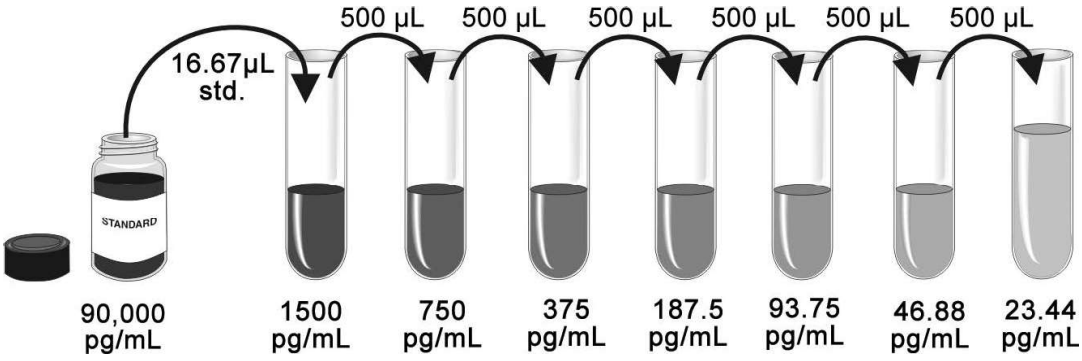
Substrate Solution **-** Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well. **Take care not to contaminate the Color Reagent. If the mixed color reagent is blue. DO NOT USE.**

Human VSIG4 Standard - Reconstitute the Human VSIG4 Standard with 1 mL of Dilution Buffer to make stock solution. Shake the vial gently until the lyophilized powder totally dissolved (**Do not turn the vial upside down**). Mix the standard to ensure complete reconstitution prior to making dilutions.

Prepare serially diluted standards as described in the following step:

Pipette 1000 µL of Dilution Buffer into the 1500 pg/mL tube. Pipette 500 µL of Dilution Buffer into the remaining tubes. Use the stock solution to produce a dilution series as the following figure. Mix each tube thoroughly before the next transfer. The 1500 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). **Ensures each assay has a standard curve. DO NOT USE the standard curve on other plates or other days.**

The following graph is only for demonstration purposes. The concentration of stock solution is lot specific and need be calculated with the actual amount of standard labeled on the standard vial.



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous

sections.

2. Remove unused microplate strips from the plate frame, return them to the foil pouchcontaining the desiccant pack, and reseal.

3. Wash each well three times with Wash Buffer (300 µL/well) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. **Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.**

4. Add 100 µL of each serially diluted protein standard or test sample per well including a zero standard. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Cover/seal the plate and incubate for 2 hours at room temperature.

5. Repeat the aspiration/wash as in Step 3.

6. Add 100 µL of Detection Antibody in working concentration to each well. Cover/seal the plate and incubate for 1 hour at room temperature.

7. Repeat the aspiration/wash as in Step 3.

8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature**. Protect from light.**

9. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. **Determine the optical density of each well within 20 minutes**, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

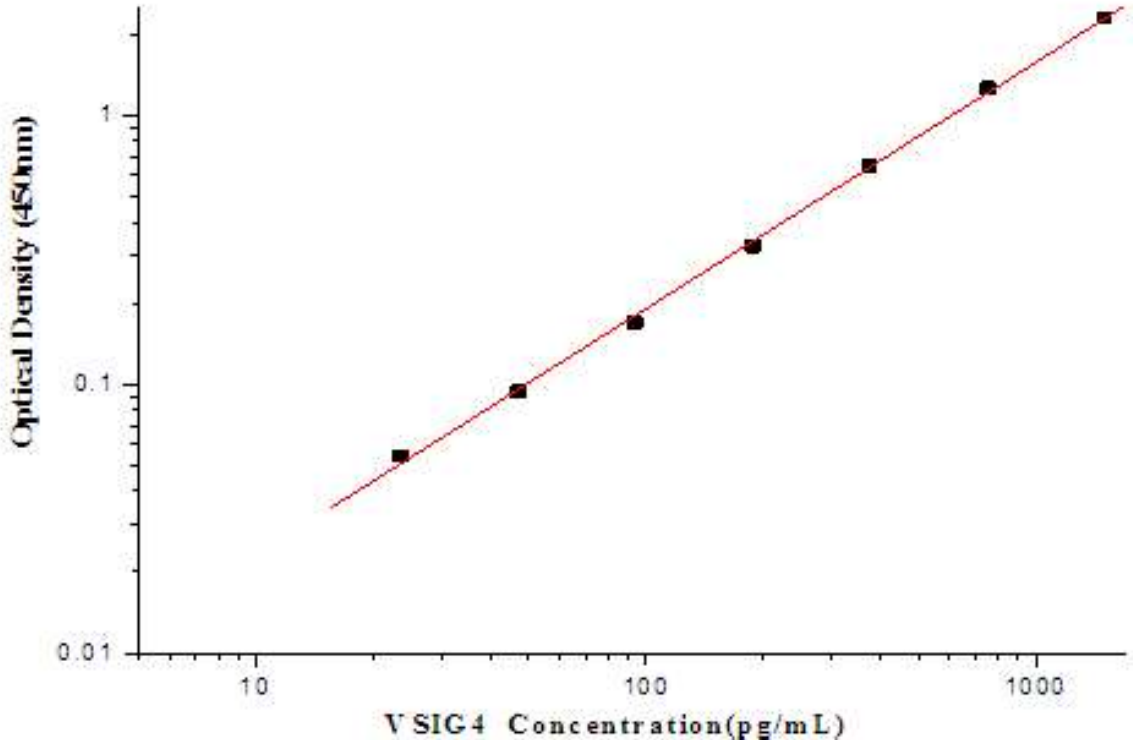
Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.) .

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. Most graphing software can help make the curve and a four parameter logistic (4-PL) usually provide the best fit, though other equations (e.g. linear, log/log) can also be tried to see which provides the most accurate. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.

Concentration (pg/mL)	Zero standard subtracted OD
0	0
23.44	0.054
46.88	0.094
93.75	0.170
187.5	0.326
375	0.647
750	1.259
1500	2.308



PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in five separate assays to assess inter-assay precision.

	Intra -assay Precision			**Inter -assay Precision**		
Sample	1	2	3	1	2	3
N	20	20	20	3	3	3
Mean (pg/mL)	207	457	867	214	467	903
SD	6.35	5.69	17.67	10.92	24.39	49.98
CV (%)	3.1%	1.2%	2.0%	5.1%	5.2%	5.5%

RECOVERY

The recovery of Human VSIG4 spiked to different levels throughout the range of the assay in related matrices was evaluated.

Sample	**Average % Recovery**	**Range**
cell culture supernates (n=4)	85	81-88%

LINEARITY

		cell culture supernates
1:2	recovery of detected	83%
1:4	recovery of detected	82%
1:8	recovery of detected	81%
1:16	recovery of detected	88%

SENSITIVITY

The minimum detectable dose (MDD) of Human VSIG4 is typically less than 12.63 pg/mL. The MDD was determined by adding three standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified HEK 293-expressed recombinant Human VSIG4.

SAMPLE VALUES

The concentration of Human VSIG4 detected in a Hela cell lysate is 6.3ng/mL.The Hela cell lysate' total protein concentration was about 9.3mg/mL.

SPECIFICITY

This assay recognizes both recombinant and natural Human VSIG4. The factors listed below were prepared at 50 ng/mL in dilution buffer and assayed for cross-reactivity. No cross-reactivity was observed.

Recombinant human			
APP	FLT4	NEF	GK
TIMP2	IL1	IL2	IL6
IL8	IL33	IL10	IL4
SCF	PDGF	TNF-α	GM-CSF
IFN-γ	VEGF	MIP-1β	TIMP1
Recombinant mouse			
VSIG4			

Preparations of APP、FLT4、NEF and GK at 25 ng/mL in a mid-range recombinant human VSIG4 control were assayed for interference. No significant interference was observed.

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃. The reconstituted standards should be aliquoted and avoid repeated freeze-thaw cycles.
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	Color Reagent should be clear and colorless prior to addition to wells	Color Reagent should be clear and colorless prior to addition to wells
	Use clean tubes and pipettes tips	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

ASSAY SUMMARY