RES101-EN.01

resDetect™ Human VCAM-1 (Fc Tag) ELISA Kit (Residue Testing)

Pack Size: 96 tests

Catalog Number: RES-A101

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedure

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Content

【Background】

Vascular cell adhesion protein 1 (VCAM1) is also known as CD106, INCAM-100 and L1CAM, is a cell surface sialoglycoprotein belonging to the immunoglobulin superfamily. VCAM1 / CD106 contains 7 Ig-like C2-type (immunoglobulin-like) domains.CD106 / VCAM1 interacts with the beta-1 integrin VLA4 on leukocytes and mediates both adhesion and signal transduction. The VCAM1 / VLA4 interaction may play a pathophysiologic role both in immune responses and in leukocyte emigration to sites of inflammation.

To support the development of CAR-T drugs, ACROBiosystems developed Human VCAM-1 (Fc Tag) ELISA Kit (Residue Testing) with rigorous methodological validation, which is used evaluation the quality of CAR-T products in drug development and CMC quality control stages. Besides, this kit can also be used for the quantitative determination of GMP human VCAM-1 Protein (ACROBiosystems, Cat#GMP-VC1H25) concentrations.

【Principle of the assay】

This assay kit is used to measure the levels of human VCAM-1 by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-Human VCAM-1 Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-Human VCAM-1 Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of human VCAM-1 bound.

【Precautions】

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Do not use reagents past their expiration date.
3. Do not mix or substitute reagents with those from other kits or other lot number kits.
4. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
5. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage. The kit is

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designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

【Material provided】

Table 1. Materials provided

ID	Component	Size (96 T)	Format	Storage	
				Unopened	Opened
RES101-C01	Pre-coated Anti-Human VCAM-1 Antibody Microplate	1 plate	Solid	2-8℃	2-8℃
RES101-C02	Human VCAM-1 (Fc Tag) Standard	15 μg	Powder	2-8℃	-70℃
RES101-C03	Biotin-Anti-Human VCAM-1 Antibody	15 μg	Powder	2-8℃	-70℃
RES101-C04	Streptavidin-HRP	50 μL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RES101-C05	10×Washing Buffer	50 mL	Liquid	2-8℃	2-8℃
RES101-C06	2×Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
RES101-C07	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RES101-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

Note: It is recommended that Streptavidin-HRP be centrifuged briefly before use to deposit liquid from the tube wall or cap to the bottom of the tube.

【Storage】

1. Unopened kit should be stored at 2℃-8℃ upon receiving.
2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.
3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

【Reagents and Consumables / Equipment needed but not supplied】

Table 2. Reagents and Consumables / Equipment needed but not supplied

Item	Specification
Deionized or distilled water	/
Single or multi-channel micropipettes	Pipettes must be calibrated
Low retention pipette tips	10 μL, 100 μL, 300 μL, 1000 μL

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Reagent bottle	500 mL
Centrifugal tube	1.5 mL, 10 mL
Microporous plate shaker	For plate shaking
Vortex	/
Timer	/
Incubator	Can be set at 37℃
Microplate reader	Single or dual wavelength microplate reader capable of measuring signals of 96-well microplates at absorbance wavelengths of 450 nm and 630 nm.

【Quick guide】

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【Reagent preparation】

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 3, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vertexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, the size of the aliquot should not be less than 5 μg.

Note: Considering inevitable minor quantitation variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for even better accuracy.

Table 3. Preparation method

ID	Components	Format (96 T)	Concentration	Reconstituted water Vol.
RES101-C02	Human VCAM-1 (Fc Tag) Standard	15 μg	150 μg/mL	100 μL
RES101-C03	Biotin-Anti-Human VCAM-1 Antibody	15 μg	150 μg/mL	100 μL

【Recommend sample preparation】

1. Working solution preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Anti-Human VCAM-1 Antibody working fluid:

Dilute Biotin-Anti-Human VCAM-1 Antibody to 0.15 μg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

1.5 Sample preparation:

If the sample to be tested is the cell supernatant, dilute test sample at 1:2 with 1×Dilution Buffer. The

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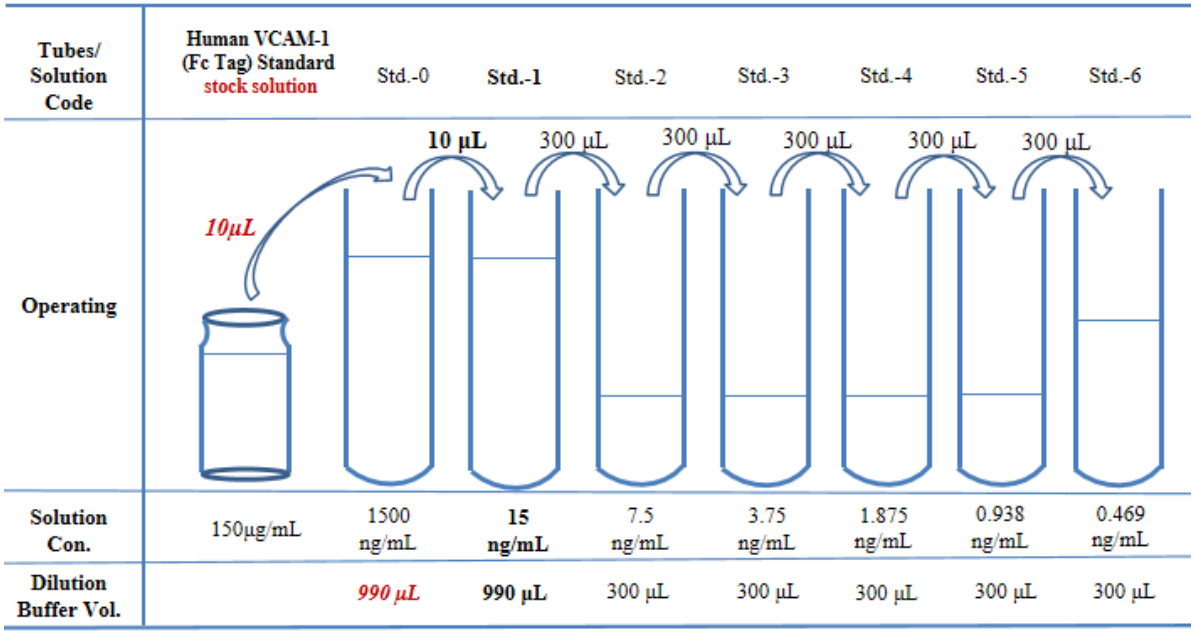
volume ratio of samples to diluent is 1:1.

2. Preparation of standard curve

The concentration of the reconstituted human VCAM-1 Calibrator (RES101-C02) is 150 μg/mL, prepare (Std.-0) by diluting 10 μL the reconstituted human VCAM-1 Calibrator into 990 μL 1×Dilution Buffer, mix gently well. Then prepare the highest concentration of standard curve, Std.-1 (15 ng/mL), by diluting 10 μL Std.-0 into 990 μL 1×Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 μL of 1×Dilution Buffer into each tube. Make sure to mix well every time. 1×Dilution Buffer serves as blank.

Figure 1. Preparation of 1:1 serial dilution of the Human VCAM-1 standard



3. Add samples

Add 100 μL Calibrator and samples to each well. For blank Control wells, please add 100 μL 1×Dilution Buffer.

Note: It is recommended to set double holes for samples and standard curves to be tested. Blank is a Blank Dilution Buffer hole.

Figure 2. This plate layout is recommended for recording standards and samples

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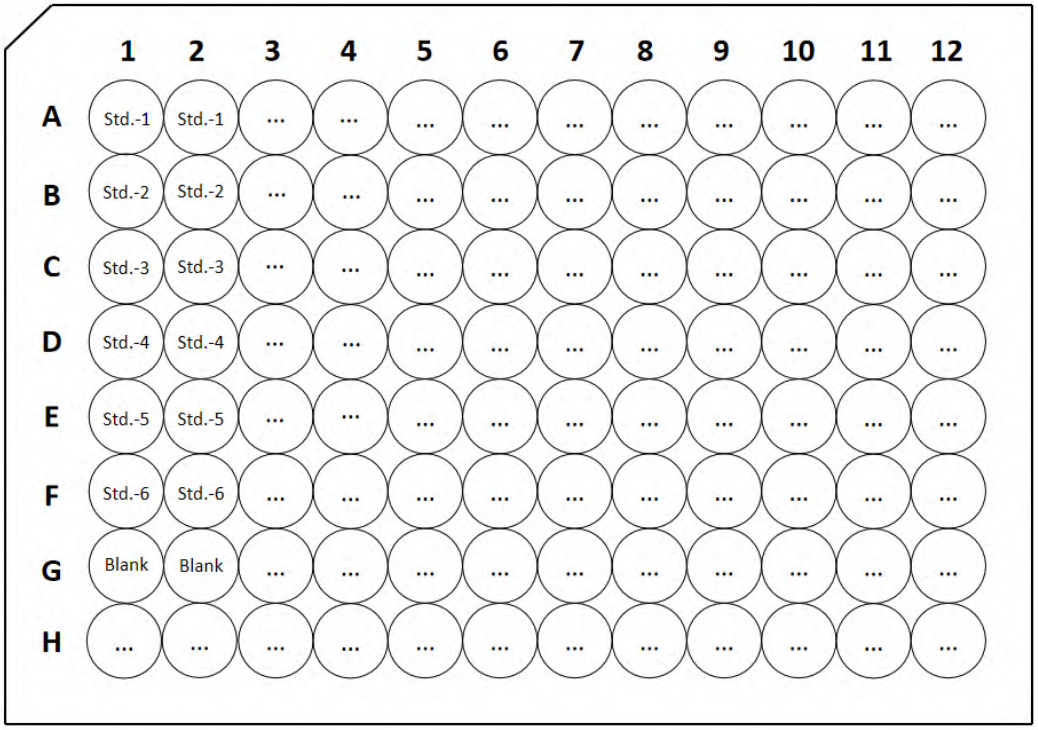

4. Incubation

Seal the plate with microplate sealing film, shake at 400 rpm, and incubate at room temperature for 1.0 hour.

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 10 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

6. Add Biotin-Anti-Human VCAM-1 Antibody

For all wells, add 100 µL Biotin-Anti-Human VCAM-1 Antibody (dilute to 0.15 µg/mL) working solution. Please prepare it for one-time use only.

7. Incubation

Seal the plate with microplate sealing film, shake at 400 rpm, and incubate at room temperature for 1.0 hour.

8. Washing

Repeat step 5.

9. Add Streptavidin-HRP

For all wells, add 100 µL Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

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10. Incubation

Seal the plate with microplate sealing film, shake at 400 rpm, and incubate at room temperature for 1.0 hour.

11. Washing

Repeat step 5.

12. Substrate reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

13. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

Note: The color in the wells should change from blue to yellow.

14. Data recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

Note: To reduce the background noise, subtract the value read at OD_{450nm} with the value read at OD_{630nm}.

【Calculation of results】

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (OD).
2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
3. Normal range of Standard curve: $R^2 \geq 0.9900$.
4. Detection range: 0.469 ng/mL-15 ng/mL. If the OD value of the sample to be tested is higher than 15 ng/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 0.469 ng/mL, the sample should be reported.

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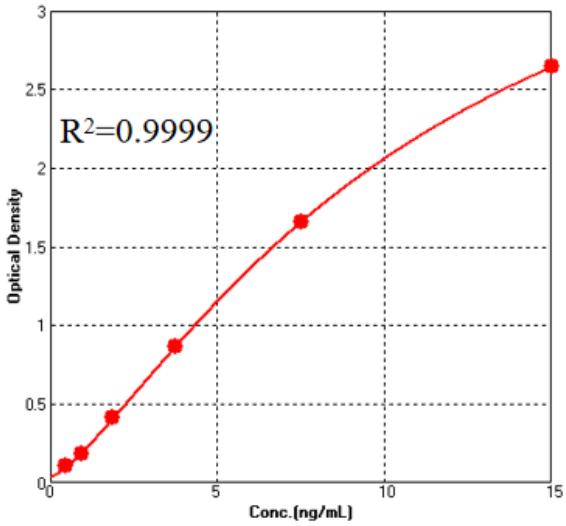
【Typical data】

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (ng/mL)	O.D.-1	O.D.-2	Average	Corrected
15	2.682	2.719	2.701	2.644
7.5	1.720	1.715	1.718	1.661
3.75	0.906	0.943	0.925	0.868
1.875	0.443	0.501	0.472	0.416
0.938	0.217	0.269	0.243	0.187
0.469	0.160	0.161	0.161	0.104
0	0.059	0.054	0.056	/



【Sensitivity】

The minimum detectable concentration of human VCAM-1 is 0.045 ng/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

【Precision】

1. **Intra-assay Precision**

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

2. **Inter-assay Precision**

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

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	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (ng/mL)	12.260	5.018	1.233	11.668	4.900	1.212
SD	0.420	0.156	0.040	0.514	0.162	0.035
CV (%)	3.4	3.1	3.2	4.4	3.3	2.9

【Recovery】

Five parts of blank T cell culture supernatant were added with different concentrations of human VCAM-1, and the T cell culture supernatant without human VCAM-1 was used as background to calculate the recovery rate. The range of recovery rate is 85.3-102.3%, and the average recovery is 94.9%.

Sample(n=5)	Average Recovery %	Range %
T cell culture supernatant (n=5)	94.9	85.3-102.3

【Linearity】

To assess the linearity of the assay, samples spiked with high concentrations of VCAM-1 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (1640)
1:2	Average Recovery (%)	99.7	99.1
	Range (%)	96.2-101.3	95.0-108.4
1:4	Average Recovery (%)	100.7	99.3
	Range (%)	94.7-108.5	94.5-109.9
1:8	Average Recovery (%)	100.8	101.4
	Range (%)	97.7-107.3	94.5-108.1
1:16	Average Recovery (%)	105.2	107.6

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	Range (%)	102.5-109.6	102.2-110.0

Note: The example data is for reference only.

【Specificity】

This assay recognizes natural and recombinant human VCAM-1. No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines.

Human			
ALCAM	ICAM-1	P-Selectin	TROP-2
Activin A	DNAM-1	E-Selectin	Fibronectin
Laminin 521 E8	Laminin 511 E8	Laminin 521	

【Interfering substances】

Verify potential matrix effects by adding different levels of DMSO and HSA to the diluted buffer.

Additive	Tolerated concentration
DMSO	20%
HSA	5%

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【Troubleshooting guide】

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again

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