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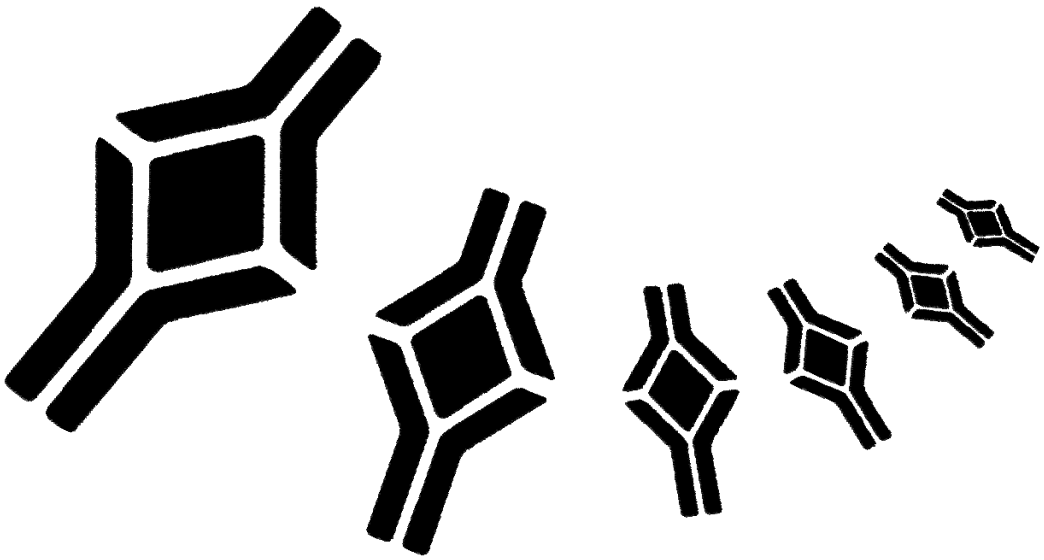

Monkey sPECAM-1 ELISA

Product Data Sheet

Cat. No.: RBMS651R

For Research Use Only

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» **Use only the current version of Product Data Sheet enclosed with the kit!**

1 INTENDED USE

The monkey sPECAM-1 ELISA is an enzyme-linked immunosorbent assay for quantitative detection of soluble monkey platelet endothelial cell adhesion molecule-1 in cell culture supernatants, monkey serum, plasma or other body fluids. **The monkey sPECAM-1 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2 SUMMARY

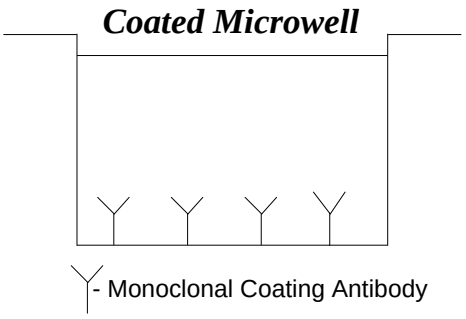
PECAM-1 (platelet endothelial cell adhesion molecule-1) also called CD31 and EndoCAM is a newly characterized adhesion molecule that belongs to the immunoglobulin superfamily [8]. PECAM-1 is a transmembrane glycoprotein with a molecular weight of approximately 130 kDa, depending on the degree of glycosylation [4].
PECAM-1 is constitutively expressed on all vascular cells and has provided a useful immunohistochemical marker of blood vessels, particularly in the setting of angiogenesis. It has also been found on platelets, monocytes, neutrophils and $CD8^+$ T cells. Bone marrow stem cells and transformed cell lines of the myeloid and megakaryocytic lineage also express PECAM-1 [4,5]. Interestingly, PECAM-1 was also detected on solid tumor lines [9].
Recent studies suggest a role for PECAM-1 in the inflammatory process and leukocyte-endothelial interaction. The process of leukocyte emigration to the site of inflammation can be dissected into three successive stages: (1) rolling, mediated by the selectins; (2) tight adhesion mediated by ICAMs and their counter-receptors, the integrins; and (3) transmigration of leukocytes through intercellular junctions of vascular endothelial cells which requires PECAM-1 [2,7,10]. PECAM-1 appears to be able to interact both with itself (homophilic binding) and with other "non-PECAM-1" molecules (heterophilic binding).

PECAM-1 is an early and sensitive marker for tumor-induced angiogenesis [1]. Several data have suggested that PECAM-1 may be involved in the process of angiogenesis in a developing vertebrate embryo [2] as well as during metastases formation [9].

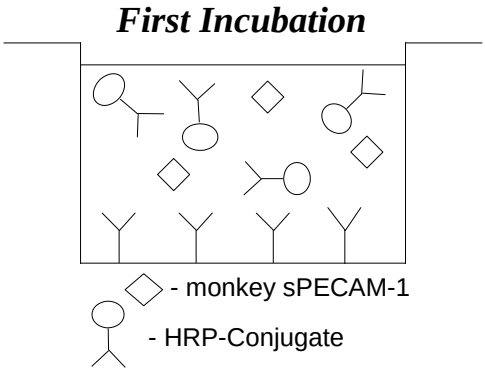
Besides the membrane-bound form of PECAM-1 a soluble form of the molecule exists, which is smaller than cell-associated PECAM-1, and contains the cytoplasmic tail. This form of soluble PECAM-1 is encoded by an alternatively spliced mRNA from which the exon containing the transmembrane domain has been removed. Soluble PECAM-1 can be detected in plasma [6].

3 PRINCIPLES OF THE TEST

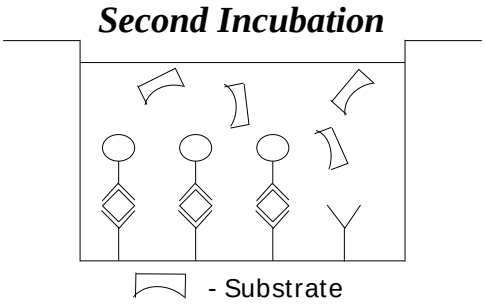
An anti-monkey-sPECAM-1 monoclonal coating antibody is adsorbed onto microwells.



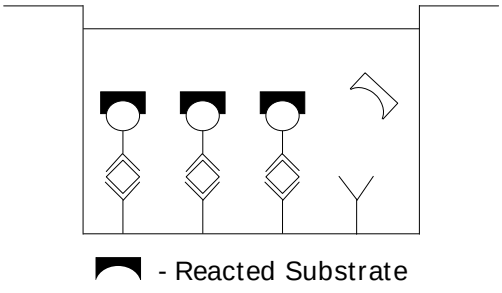
Monkey sPECAM-1 present in the sample or standard binds to antibodies adsorbed to the microwells; a HRP-conjugated monoclonal anti-monkey-sPECAM-1 antibody is added and binds to monkey sPECAM-1 captured by the first antibody.



Following incubation unbound enzyme conjugated anti-monkey-sPECAM-1 is removed during a wash step and substrate solution reactive with HRP is added to the wells.



A coloured product is formed in proportion to the amount of soluble monkey PECAM-1 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven monkey sPECAM-1 standard dilutions and monkey sPECAM-1 sample concentration determined.



4 REAGENTS PROVIDED

1 aluminium pouch with a **Antibody Coated Microtiter Strips with** Monoclonal Antibody (murine) to monkey sPECAM-1

1 vial (5 µl) **HRP-Conjugate** anti-monkey-sPECAM-1 monoclonal (murine) antibody

2 vials (60 U/ml) **monkey sPECAM-1 Standard**

1 bottle (50 ml) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20)

1 vial (5 ml) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20 and 10% BSA)

1 bottle (12 ml) **Sample Diluent** (buffered protein matrix)

1 vial (7 ml) **Substrate Solution I** (tetramethyl-benzidine)

1 vial (7 ml) **Substrate Solution II** (0.02 % buffered hydrogen peroxide)

1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)

1 vial (0.4 ml) **Blue-Dye**

1 vial (0.4 ml) **Green-Dye**

2 adhesive **Plate Covers**

Reagent Labels

5 STORAGE INSTRUCTIONS

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.
The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

6 SPECIMEN COLLECTION

Cell culture supernatants, monkey serum, plasma or other body fluids are suitable for use in the assay. Remove serum or plasma from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive monkey sPECAM-1. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

For stability of samples refer to 13. E.

7 MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes

- 5 µl to 1,000 µl adjustable single channel micropipettes with disposable tips

- 50 µl to 300 µl adjustable multichannel micropipette with disposable tips

- Multichannel micropipette reservoir

- Beakers, flasks, cylinders necessary for preparation of reagents

- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

- Glass-distilled or deionized water

- Statistical calculator with program to perform linear regression analysis

8 PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.

- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.

- Do not mix or substitute reagents with those from other lots or other sources.

- Do not use kit reagents beyond expiration date on label.

- Do not expose kit reagents to strong light during storage or incubation.

- Do not pipette by mouth.

- Do not eat or smoke in areas where kit reagents or samples are handled.

- Avoid contact of skin or mucous membranes with kit reagents or specimens.

- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.

- Reagents containing thimerosal as preservative may be toxic if ingested.

- Avoid contact of substrate solutions with oxidizing agents and metal.

- Avoid splashing or generation of aerosols.

- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.

- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.

- Exposure to acids will inactivate the conjugate.

- Glass-distilled or deionized water must be used for reagent preparation.

- Substrate solutions must be at room temperature prior to use.

- Decontaminate and dispose specimens and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.

- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 PREPARATION OF REAGENTS

Except for the HRP-Conjugate (reagent C.) and the TMB Substrate Solution (reagent D.), the reagents should be prepared before starting with the test procedure.

A. Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

B. Assay Buffer

Mix the contents of the bottle well. Add contents of **Assay Buffer Concentrate** (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days. Assay Buffer may be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. Preparation of HRP-Conjugate

Dilute the **HRP-Conjugate** 1:25 just prior to use by adding 120 µl **Assay Buffer** (reagent B.) to the tube containing the HRP-Conjugate concentrate. Mix the contents of the tube well.
Make a further 1:100 dilution with **Assay Buffer** (reagent B) in a clean plastic tube.

Please note that the HRP-Conjugate should be used within 30 minutes after dilution or stored at -20°C in prediluted form up to one month. The second dilution (1:100) of the HRP-Conjugate may be prepared as needed according to the following table:

Number of Strips	prediluted (1:25) HRP-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. TMB Substrate Solution

Using clean pipettes and containers known to be metal free, dispense an equal volume of **Substrate Solution I** into **Substrate Solution II** and swirl gently to mix. The TMB Substrate Solution may develop a yellow tinge over time. This does not seem to affect product performance. A blue colour present in the TMB Substrate Solution, however, indicates that it has been contaminated and must be discarded. The TMB Substrate Solution must be used within a few minutes after mixing. Warm to room temperature before use. Avoid direct exposure of TMB reagents to intense light and oxidizing agents during storage or incubation.

Substrate preparation by assay size:

Number of Strips	Substrate Solution I (ml)	Substrate Solution II (ml)
1 - 6	3.0	3.0
1 - 12	6.0	6.0

E. Addition of colour-giving reagents: Blue-Dye, Green-Dye

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.
Alternatively, the dye solutions from the stocks provided (***Blue-Dye, Green-Dye***) can be added to the reagents according to the following guidelines:

1. Diluent: Before sample dilution add the ***Blue-Dye*** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of ***Blue-Dye***, proceed according to the instruction booklet.

5 ml Diluent	20 µl ***Blue-Dye***
12 ml Diluent	48 µl ***Blue-Dye***

2. HRP-Conjugate: Before dilution of the concentrated conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet, preparation of HRP-conjugate.

3 ml Assay Buffer	30 µl ***Green-Dye***
6 ml Assay Buffer	60 µl ***Green-Dye***
12 ml Assay Buffer	120 µl ***Green-Dye***

a. Mix all reagents thoroughly without foaming before use.

b. Determine the number of Microwell Strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank, and optional control sample should be assayed in duplicate. Remove extra **Microwell Strips coated with Monoclonal Antibody** (murine) to monkey sPECAM-1 from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

c. Wash the microwell strips twice with approximately 300 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.

   After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

d. Add 100 µl of **Sample Diluent** in duplicate to all standard wells. Prepare standard dilutions by pipetting 100 µl of **monkey sPECAM-1 Standard**, in duplicate, into well A1 and A2 (see Figure 1 and 2). Mix the contents by repeated aspiration and ejection and transfer 100 µl to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of monkey sPECAM-1 standard dilutions ranging from 30 to 0.48 U/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of monkey sPECAM-1 standard dilutions:

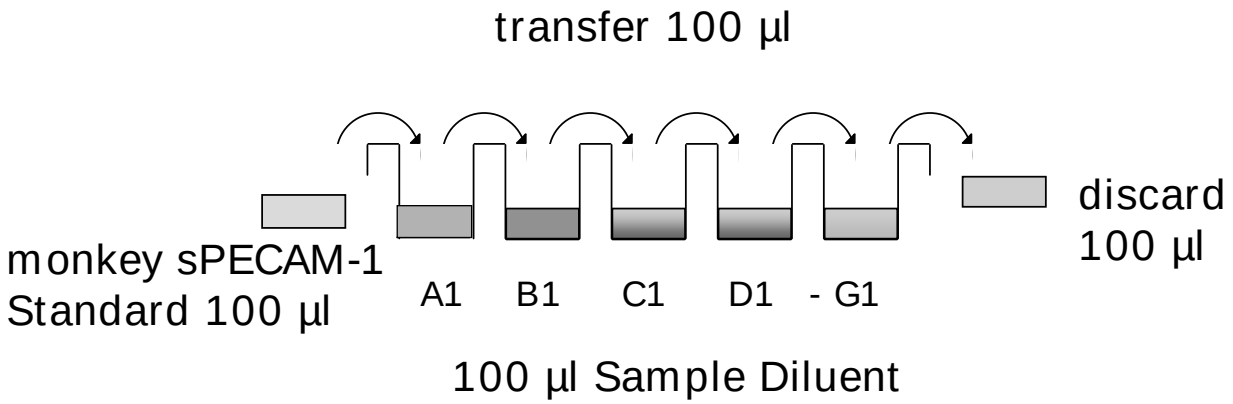


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (30 U/ml)	Standard 1 (30 U/ml)	Sample 1	Sample 1
B	Standard 2 (15 U/ml)	Standard 2 (15 U/ml)	Sample 2	Sample 2
C	Standard 3 (7.5 U/ml)	Standard 3 (7.5 U/ml)	Sample 3	Sample 3
D	Standard 4 (3.75 U/ml)	Standard 4 (3.75 U/ml)	Sample 4	Sample 4
E	Standard 5 (1.9 U/ml)	Standard 5 (1.9 U/ml)	Sample 5	Sample 5
F	Standard 6 (0.95 U/ml)	Standard 6 (0.95 U/ml)	Sample 6	Sample 6
G	Standard 7 (0.48 U/ml)	Standard 7 (0.48 U/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

e. Add 100 µl of **Sample Diluent** in duplicate to the blank wells.

f. Add 90 µl of **Sample Diluent** to all wells designated for samples.

g. Add 10 µl of each **Sample**, in duplicate, to the designated wells and mix the contents.

h. Prepare **HRP-Conjugate** (Refer to preparation of reagents 9.C.).

i. Add 50 µl of diluted **HRP-Conjugate** to all wells, including the blank wells.

j. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 3 hours, if available on a rotator set at 100 rpm.

k. Prepare **TMB Substrate Solution** a few minutes prior to use (Refer to preparation of reagents 9.D.).

l. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

m. Pipette 100 µl of mixed **TMB Substrate Solution** to all wells, including the blank wells.

n. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes, if available on a rotator set at 100 rpm. Avoid direct exposure to intense light.
The colour development on the plate should be monitored and the substrate reaction stopped (see point o. of this protocol) before positive wells are no longer properly recordable.
It is recommended to add the stop solution when the highest standard has developed a dark blue colour.
Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as an OD of 0.6 – 0.65 is reached.

o. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

p. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the monkey sPECAM-1 standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

11 CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicatestandards and samples. Duplicates should be within 20 per cent of themean.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the monkey sPECAM-1 concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating monkey sPECAM-1 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding monkey sPECAM-1 concentration.

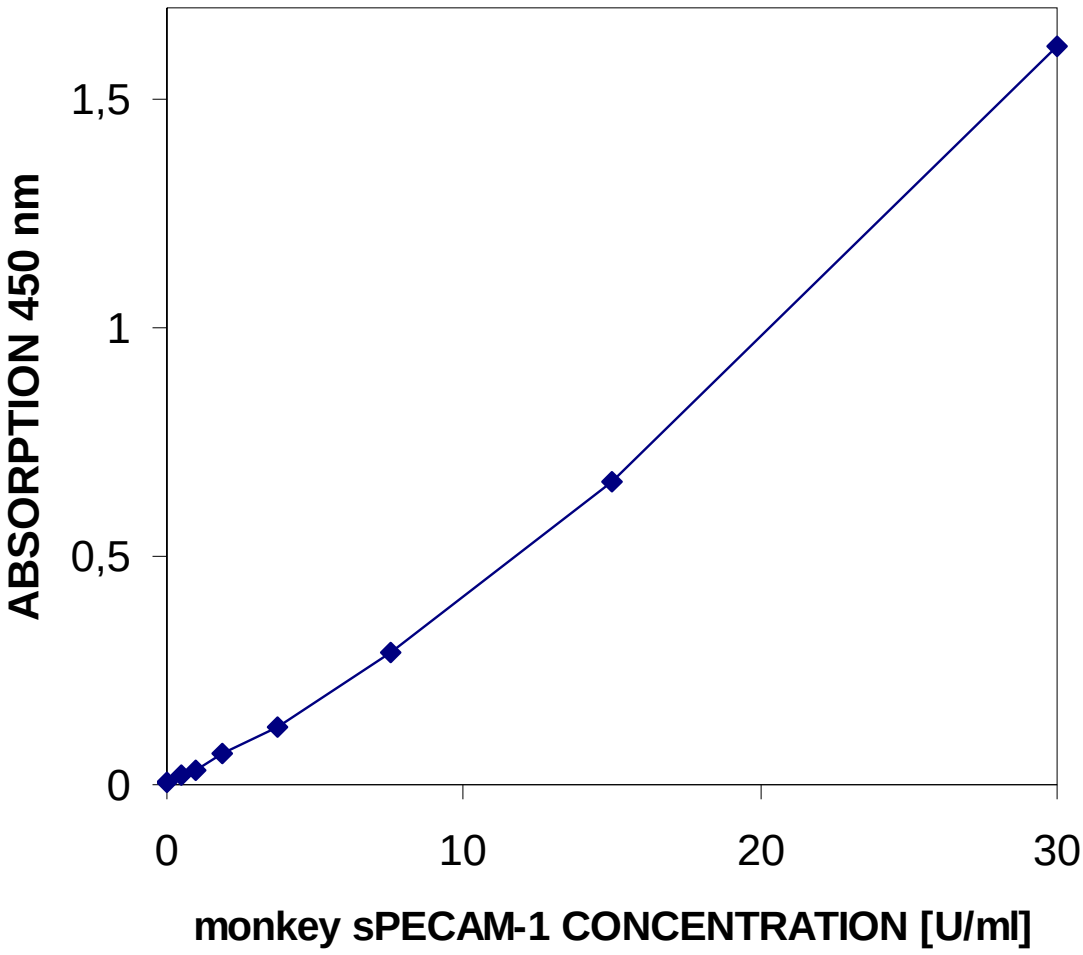
- **For samples which have been diluted according to the instructions given in this manual 1:10 within the microwells, the concentration read from the standard curve must be multiplied by the dilution factor (x 10).**

- **Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low monkey sPECAM-1 levels. Such samples require further dilution of 1:20 - 1:40 with Sample Diluent in order to precisely quantitate the actual monkey sPECAM-1 level.**

- It is suggested that each testing facility establishes a control sample of known monkey sPECAM-1 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

- A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for the monkey sPECAM-1 ELISA. Monkey sPECAM-1 was diluted in serial two-fold steps in Sample Diluent, symbols represent the mean of three parallel titrations.
Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.



Typical data using the monkey sPECAM-1 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	monkey sPECAM-1 Concentration (U/ml)	O.D. Mean	C.V. (%)
1	30	1.618	2.8
	30		
2	15	0.661	6.8
	15		
3	7.5	0.287	5.4
	7.5		
4	3.75	0.126	0.5
	3.75		
5	1.9	0.066	7.7
	1.9		
6	0.95	0.033	5.4
	0.95		
7	0.48	0.022	7.4
	0.48		
Blank	0	0.007	
	0		

12 LIMITATIONS

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.

- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.

- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

13 PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection of monkey sPECAM-1 defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 0.06 U/ml (mean of 6 independent assays).

B. Reproducibility

a. Intra-assay

Reproducibility within the assay was evaluated in independent experiments. The overall Intra-assay coefficient of variation has been calculated to be 2 %.

b. Inter-Assay

Assay to assay reproducibility within one laboratory was evaluated in independent experiments by three technicians. The overall inter-assay coefficient of variation has been calculated to be <10 %.

C. Spiking Recovery

Spiking samples were prepared by adding four different levels of monkey sPECAM-1 to a monkey serum sample. The overall mean recovery was 110 %.

D. Dilution Linearity

Four serum samples with different levels of monkey sPECAM-1 were assayed at four serial two-fold dilutions (1:10 - 1:80) with 4 replicates each. The overall mean recovery was 105 %.

E. Sample Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked with monkey sPECAM-1) were stored at -20°C and thawed several times, and monkey sPECAM-1 levels determined. There was no significant loss of monkey sPECAM-1 concentration between 0 and 5 freeze-thaw cycles.

F. Sample Storage Stability

Aliquots of a serum sample (unspiked or spiked with monkey sPECAM-1) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the monkey sPECAM-1 level determined after 24 hours. There was no significant loss of monkey sPECAM-1 immunoreactivity during storage under above conditions.

14 REFERENCES

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Science 262, 1580-1582.

15 REAGENT PREPARATION SUMMARY

A. Wash Buffer

Add **Wash Buffer Concentrate** 20 x (50 ml) to 950 ml distilled water

B. Assay Buffer

Number of Strips	Assay Buffer Concentr. (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. HRP-Conjugate

Add 120 µl **Assay Buffer** to the tube containing HRP-Conjugate concentrate. Mix. Make further dilution according to table.

Number of Strips	prediluted (1:25) HRP-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. TMB Substrate Solution

Number of Strips	Substrate Solution I (ml)	Substrate Solution II (ml)
1 - 6	3.0	3.0
1 - 12	6.0	6.0

16 TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Sample Diluent**, in duplicate, to standard wells
- Pipette wells 100 µl **monkey sPECAM-1 Standard** into the first wells and create standard dilutions ranging from 30 to 0.48 U/ml by transferring 100 µl from well to well. Discard 100 µl from the last wells
- Add 100 µl **Sample Diluent**, in duplicate, to the blank wells
- Add 90 µl **Sample Diluent** to the sample wells
- Add 10 µl **Sample**, in duplicate, to designated wells
- Prepare **HRP-Conjugate**
- Add 50 µl of diluted **HRP-Conjugate** to all wells
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C)
- Prepare **TMB Substrate Solution** few minutes prior to use
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of mixed **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for about 15 minutes at room temperature (18°to 25°C).
- Add 100 µl **Stop Solution** to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

Note: For samples which have been diluted according to the instructions given in this manual 1:10, the concentration read from the standard curve must be multiplied by the dilution factor (x 10). Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low monkey sPECAM-1 levels. Such samples require further dilution of 1:20 - 1:40 with Sample Diluent in order to precisely quantitate the actual monkey sPECAM-1 level.

NOTES

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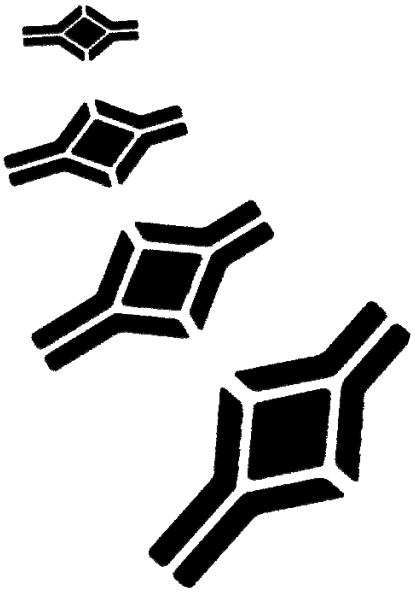

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