PRODUCT INFORMATION & MANUAL

Human sCD105 Instant ELISA

BMS2105INST

Enzyme-linked immunosorbent assay for quantitative detection of human sCD105.

For research use only.
Not for diagnostic or therapeutic procedures.
128 Tests

eBioscience®
An Affymetrix Company

Human sCD105 Instant ELISA

North America

Technical Support:

Research Products:
888.810.6168
858.642.2058
tech@eBioscience.com

Clinical Products:
877.726.8559
858.642.2058
tech@eBioscience.com

Customer Service:
888.999.1371
858.642.2058
info@eBioscience.com

Fax:
858.642.2046

Europe/International*

Technical Support:
+43 1 796 40 40-120
tech@eBioscience.com

Customer Service:
+43 1 796 40 40-304
europe@eBioscience.com

Fax:
+43 1 796 40 40-400

Bender MedSystems GmbH
Campus Vienna Biocenter 2
1030 Vienna, Austria
www.eBioscience.com

* Customers outside North America and Europe may contact their eBioscience distributor listed on our website at www.eBioscience.com/distributors.

TABLE OF CONTENTS

1 Intended Use

The human sCD105 Instant ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human sCD105. The human sCD105 Instant ELISA is **for research use only. Not for diagnostic or therapeutic procedures.**

2 Summary

Endoglin, also known as CD105, is a 180 kDa homodimeric co-receptor for members of the TGF-beta superfamily. It is expressed on the surface of endothelial cells, subsets of bone marrow cells (erythroid cell precursors) activated macrophages, fibroblasts, human chondrocytes and smooth muscle cells and profibrogenic liver cells.

A major role for endoglin is in regulating transforming growth factor-β-dependent vascular remodeling and angiogenesis. Endoglin expression increases during angiogenesis, wound healing, and inflammation, all of which are associated with TGFβ signaling and alterations in vascular structure. Endoglin binds two isoforms of TGF-beta, TGF-beta 1 and TGF-beta 3 in combination with the signaling complex of TGFβ receptors types I and II. It also is associated with the regulation of endothelial nitric oxide synthase (eNOS) activity.

Proteolytic cleavage of the extracellular domain of endoglin gives rise to soluble CD105 (sCD105), which functions to neutralize TGF-β signaling but enhances signaling pathways involving BMP7 and SMAD1, SMAD5. Disorders in the expression of Endoglin and circulating levels of sCD105 are linked to the several disease states. Experiments in healthy nulliparous women revelaed that plasma levels of soluble CD105 (sCD105) seem to be promising as an accurate marker to herald pre-eclampsia appearance, thus allowing early diagnosis and preventive therapy. Data also indicate that the phenotype of proliferating endothelial cells of Gorham disease is similar to that of the endothelial lining of vessels of metabolically active bone characterised by high expression of CD105, while that of conventional haemangioma is more similar to that of metabolically quiescent bone tissue, such as fatty marrow, with low levels of expression of CD105. It is primarily a marker of endothelial cells expressed at particularly elevated levels in angiogenic areas such as in the placenta, tissue regeneration after

injury, and tumor growth, including prostate cancer. This may have potential therapeutic and diagnostic applications. On the other hand, endoglin is rarely detected in normal epitheliums, with the exception of the inner layers of the capillaries that perform the first steps in filtering blood from the urine in human kidneys and melanocytes.

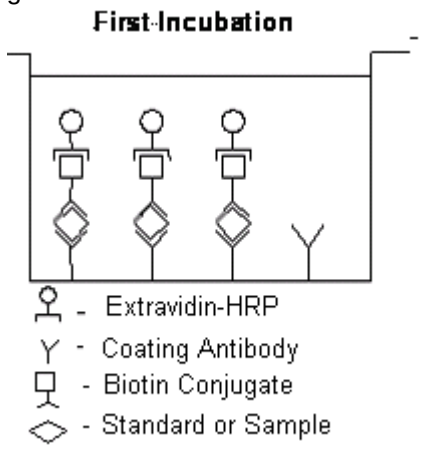
More prospective studies are necessary to confirm the effective diagnostic value of this biomarker.

For literature update refer to **www.eBioscience.com**

3 Principles of the Test

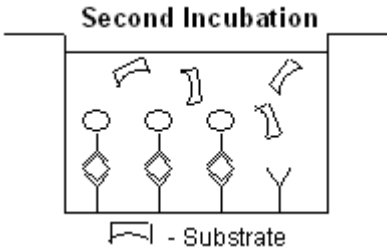
An anti-human sCD105 monoclonal coating antibody is adsorbed onto microwells. Human sCD105 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated anti-human sCD105 antibody binds to human sCD105 captured by the first antibody. Extravidin-HRP binds to the biotin conjugated anti-human sCD105 antibody.

Figure 1



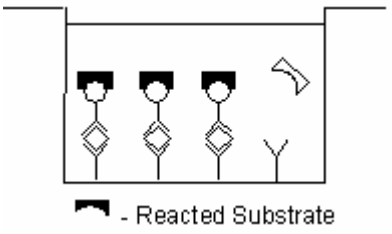
Following incubation unbound biotin conjugated anti-human sCD105 and Extravidin-HRP is removed during a wash step and substrate solution reactive with HRP is added to the wells.

Figure 2



A coloured product is formed in proportion to the amount of soluble human sCD105 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven human sCD105 standard dilutions and human sCD105 sample concentration determined.

Figure 3



4 Reagents Provided

1 aluminium pouch with **a Microwell Plate coated with monoclonal antibody** to human sCD105, Biotin-Conjugate (anti-human sCD105 monoclonal antibody), **Extravidin-HRP** and Assay Buffer, lyophilized

2 aluminium pouches with a **human sCD105 Standard curve (coloured)**

1 bottle (25 ml) **Wash Buffer Concentrate** 20x
(phosphate-buffered saline with 1% Tween 20)

1 vial (5 ml) **Assay Buffer Concentrate** 20x
(Use when an external predilution of the samples is needed)

1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)

1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)

2 adhesive **Plate Covers**

5 Storage Instructions

Store ELISA plate and Standard curves or whole kit at -20°C. The plate and the standard curves can also be removed, stored at -20°C, remaining kit reagents can be stored between 2° and 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, human serum and plasma (citrate, EDTA, heparin) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove the serum or plasma from the clot or red cells 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.

Samples must be stored frozen at -20°C to avoid loss of bioactive human sCD105. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13.5).
Avoid repeated freeze-thaw cycles. Prior to assay, frozen serum or plasma should be brought to room temperature slowly and mixed gently.

7 Materials Required But Not Provided

- 5 ml and 10 ml graduated pipettes
- 5 µl to 1000 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 shaker
- Microwell strip 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.
- Avoid contact of substrate solution 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 substrate reagent.

- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as 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 and Samples

Buffer concentrate should be brought to room temperature and diluted before starting the test procedure. If crystals have formed in the buffer concentrate, warm it gently until crystals have completely dissolved.

9.1 Wash Buffer (1x)

Pour entire contents (25 ml) of the Wash Buffer Concentrate (20x) into a clean 500 ml graduated cylinder. Bring to final volume to 500 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.

9.2 Assay Buffer (1x)

Pour the entire contents (5ml) of the **Assay Buffer Concentrate** (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently.

10 Test Protocol

- **Use plate immediately after removal from -20°C!**
- **Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water!**
- **Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results**
- **Perform the washing step with at least 400 µl of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results**
- **Allow the washing buffer to sit in the wells for a few seconds before aspiration**
- **Remove covers of the standard strips carefully in order that all the lyophilised pellets remain in the wells**

a. Prepare your samples before starting with the test procedure. Dilute samples 1:50 with **Assay Buffer** (1x) according to the following dilution scheme:
10 µl sample + 490 µl Assay Buffer (1x)

b. Determine the number of microwell Strips required to test the desired number of samples plus microwell Strips for blanks and standards (coloured). Each sample, standard, blank, and optional control sample should be assayed in duplicate. Remove extra microwell Strips from holder and store in foil bag with the desiccant provided at -20°C sealed tightly. Place microwell strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1).

c. Add **distilled water** to all **standard and blank wells** as indicated on the label of the standard strips (A1, A2 to H1, H2).

d. Add 100 µl of **distilled water** to the **sample wells**.

Table 1

Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (1000.0 pg/ml)	Standard 1 (1000.0 pg/ml)	Sample 1	Sample 1
B	Standard 2 (500.0 pg/ml)	Standard 2 (500.0 pg/ml)	Sample 2	Sample 2
C	Standard 3 (250.0 pg/ml)	Standard 3 (250.0 pg/ml)	Sample 3	Sample 3
D	Standard 4 (125.0 pg/ml)	Standard 4 (125.0 pg/ml)	Sample 4	Sample 4
E	Standard 5 (62.5 pg/ml)	Standard 5 (62.5 pg/ml)	Sample 5	Sample 5
F	Standard 6 (31.3 pg/ml)	Standard 6 (31.3 pg/ml)	Sample 6	Sample 6
G	Standard 7 (15.6 pg/ml)	Standard 7 (15.6 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

e. Add 50 µl of each **sample**, in duplicate, to the designated wells and mix the contents.

f. Cover with a Plate Cover and incubate at room temperature (18ºC to 25ºC) for 3 hours on a microplate shaker. **(Shaking is absolutely necessary for an optimal test performance.)**

g. Remove **Plate Cover** and empty wells. Wash the microwell strips 6 times with approximately 400 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, 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 no longer than 15 minutes. Do not allow wells to dry.

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

i. Incubate the microwell strips at room temperature (18° to 25°C) for 30 minutes. Avoid direct exposure to intense light.

   The colour development on the plate should be monitored and the substrate reaction stopped (see point j. of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.
   It is recommended to add the stop solution when the highest standard has developed a dark blue colour. The colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

j. 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.

k. 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 human sCD105 standards.

Note: Shaking is absolutely necessary for an optimal test performance.

11 Calculation of Results

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

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human sCD105 concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating human sCD105 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 human sCD105 concentration.

- **If instructions in this protocol have been followed samples have been diluted 1:100 (predilution: 10 µl sample + 490 µl Assay Buffer, on the plate: 50 µl sample + 50 µl Assay Buffer). The concentration read from the standard curve must be multiplied by the dilution factor (x 100).**

- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human sCD105 levels. Such samples require further external predilution according to expected human sCD105 values with Assay Buffer in order to precisely quantitate the actual human sCD105 level.**

- It is suggested that each testing facility establishes a control sample of known human sCD105 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

- A representative standard curve is shown in Figure 4. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

* N.B: There is a common dilution factor for samples due to the conjugate which must then be included in the calculation. The samples contribute 100 µl to the final volume per well. These 100 µl are composed of 50 µl of Assay Buffer plus 50 µl of the prediluted sample. This is a 1:2 dilution.
The remaining 50 µl to give 150 µl are due to the addition of 50 µl conjugate to all wells.
50 µl Assay Buffer and 50 µl conjugate results in 100 µl reconstitution volume, addition of 50 µl prediluted sample (predilution: 10 µl sample + 490 µl Assay Buffer; on the plate: 50 µl sample + 50 µl Assay Buffer = 1:100 dilution).

Figure 4

Representative standard curve for human sCD105 Instant ELISA. Human sCD105 was diluted in serial 2-fold steps in Assay Buffer. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

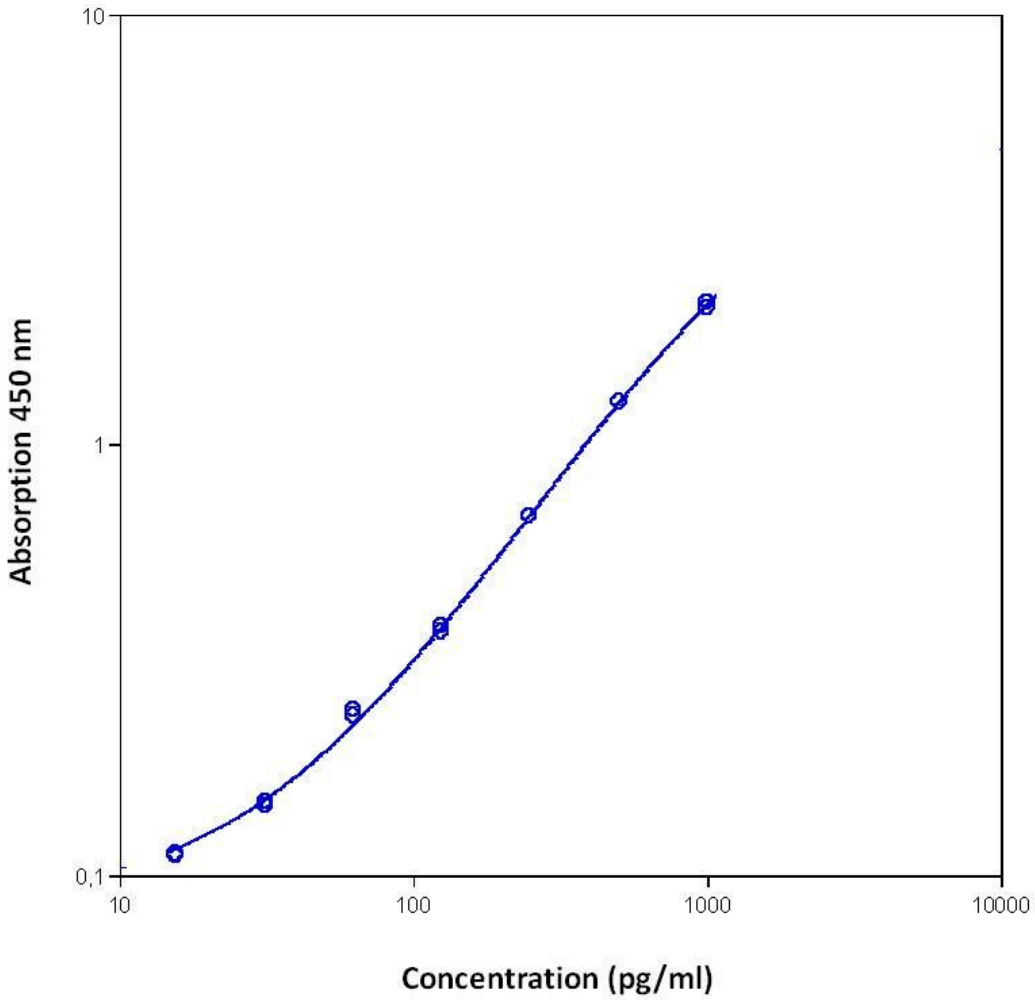


Table 2
Typical data using the human sCD105 INSTANT ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	human sCD105 Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	1000.0	2.158	2.122	1.7
		2.087		
2	500.0	1.256	1.257	0.1
		1.258		
3	250.0	0.687	0.685	0.2
		0.684		
4	125.0	0.368	0.375	1.9
		0.382		
5	62.5	0.242	0.238	1.6
		0.235		
6	31.3	0.148	0.147	0.6
		0.146		
7	15.6	0.112	0.111	0.4
		0.111		
Blank	0.0	0.070	0.069	1.5
		0.068		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

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. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the Sample.

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of human sCD105 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 2.60 pg/ml (mean of 4 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different levels of human sCD105. 2 standard curves were run on each plate. Data below show the mean human sCD105 concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 4.6%.

Table 3

The mean human sCD105 concentration and the coefficient of variation for each sample.

Positive Sample	Experiment	Human sCD105 Concentration (pg/ml)	Coefficient of Variation (%)
1	1	9806.54	4.0
	2	10851.03	5.4
	3	10754.36	7.0
2	1	6530.84	2.4
	2	7125.54	2.1
	3	6896.47	4.7
3	1	9132.45	2.8
	2	9018.96	4.2
	3	9079.05	4.2
4	1	9496.06	2.9
	2	9983.02	3.8
	3	9372.19	7.3
5	1	10083.33	7.8
	2	10425.32	3.8
	3	10382.65	4.9
6	1	10824.66	2.5
	2	11101.88	4.0
	3	10882.59	3.7
7	1	9420.44	3.6
	2	9973.70	4.6
	3	9495.92	8.9
8	1	8892.53	3.7
	2	10100.01	5.9
	3	9757.72	5.2

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human sCD105. 2 standard curves were run on each plate. Data below show the mean human sCD105 concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 3.3%.

Table 4
The mean human sCD105 concentration and the coefficient of variation of each sample

Sample	Mean human sCD105 Concentration (pg/ml)	Coefficient of Variation (%)
1	10470.64	5.5
2	6850.95	4.4
3	9076.82	0.6
4	9617.09	3.4
5	10297.10	1.8
6	10936.38	1.3
7	9630.02	3.1
8	9454.05	6.6

13.3 Spike Recovery

The spike recovery was evaluated by spiking 3 levels of human sCD105 into serum, plasma (EDTA, citrate, heparin) and cell culture supernatant. Recoveries were determined with 4 replicates each. The amount of endogenous human sCD105 in unspiked samples was subtracted from the spike values.

For recovery data see Table 5.

Table 5

Sample matrix	Spike high		Spike medium		Spike low	
	Mean (%)	Range (%)	Mean (%)	Range (%)	Mean (%)	Range (%)
Serum	90	85 – 95	99	87 – 109	107	78 – 133
Plasma (EDTA)	92	77 – 109	92	77 – 109	107	78 – 125
Plasma (citrate)	98	89 – 105	108	98 – 116	92	72 – 124
Plasma (heparin)	87	78 – 91	99	92 – 105	104	98 – 116
Cell culture supernatant	90	-	80	-	71	-

13.4 Dilution Parallelism

Serum, plasma (EDTA, citrate, heparin) and cell culture supernatant samples with different levels of human sCD105 were analysed at serial 2-fold dilutions with 4 replicates each.

For data see Table 6.

Table 6

Sample matrix	Recovery of Exp. Val.		
	Range (%)	Mean (%)	Range (%)
Serum	1:4 1 :8 1 :16	98 89 82	94 – 104 71 – 100 71 – 101
Plasma (EDTA)	1:4 1 :8 1 :16	97 94 91	94 – 102 86 – 101 73 – 109
Plasma (citrate)	1:4 1 :8 1 :16	98 94 86	83 – 112 73 – 109 70 – 101
Plasma (heparin)	1:4 1 :8 1 :16	100 102 87	94 – 110 95 – 114 81 – 96
Cell culture supernatant	1:4 1 :8 1 :16	82 80 80	- - -

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed 3 times, and the human sCD105 levels determined. There was no significant loss of human sCD105 immunoreactivity detected by freezing and thawing.

13.5.2 Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C , room temperature (RT) and 37°C, and the human sCD105 level determined after 24 h. There was no significant loss of human sCD105 immunoreactivity detected during storage under above conditions.

13.6 Specificity

A panel of 72 cytokines at 100 ng/mL was evaluated for cross-reactivity in this assay. No cross-reactivity was observed to any sample.

13.7 Expected values

Panels of 40 serum as well as plasma (EDTA, citrate, heparin) samples from randomly selected apparently healthy donors (males and females) were tested for human sCD105.
For detected human sCD105 levels see Table 7:

Table 7

Sample Matrix	Number of Samples Evaluated	Mean of Detectable (pg/ml)	Range (pg/ml)
Serum	40	6741	3692 – 12972
Plasma (EDTA)	40	5500	3385 – 9168
Plasma (Citrate)	40	4754	1899 – 10822
Plasma (Heparin)	40	6368	3492 – 10488

14 Ordering Information

North America

Technical Support:

Research Products:
888.810.6168
858.642.2058
tech@eBioscience.com

Clinical Products:
877.726.8559
858.642.2058
tech@eBioscience.com

Customer Service:
888.999.1371
858.642.2058
info@eBioscience.com

Fax:
858.642.2046

Europe/International*

Technical Support:
+43 1 796 40 40-120
tech@eBioscience.com

Customer Service:
+43 1 796 40 40-304
europe@eBioscience.com

Fax:
+43 1 796 40 40-400

Bender MedSystems GmbH
Campus Vienna Biocenter 2
1030 Vienna, Austria
www.eBioscience.com

* Customers outside North America and Europe may contact their eBioscience distributor listed on our website at www.eBioscience.com/distributors.

15 Reagent Preparation Summary

15.1 Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20x (25 ml) to 475 ml distilled water

15.2 Assay Buffer (1x)

Add **Assay Buffer Concentrate** 20x (5 ml) to 95 ml distilled water

16 Test Protocol Summary

- Place standard strips in position A1/A2 to H1/H2.
- Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 100 µl distilled water to sample wells.
- Add 50 µl sample to designated wells.
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C) on a microplate shaker. **(Shaking is absolutely necessary for an optimal test performance).**
- Empty and wash microwell strips 6 times with 400 µl Wash Buffer.
- Add 100 µl of TMB Substrate Solution to all wells including blank wells.
- Incubate the microwell strips for 30 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: Samples have been diluted 1:100 (predilution: 10 µl sample + 490 µl Assay Buffer; on the plate: 50 µl sample + 50 µl Assay Buffer), thus the concentration read from the standard curve must be multiplied by the dilution factor (x 100).