PRODUCT INFORMATION AND MANUAL

Human TIE-2 ELISA

BMS2042
and
BMS2042TEN

Enzyme-linked immunosorbent assay for quantitative

detection of human TIE-2.

For research use only.

Not for diagnostic or therapeutic procedures.

Human TIE-2
BMS2042
BMS2042TEN

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1 Intended Use

The human TIE-2 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human TIE-2. **The human TIE-2 ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

2 Summary

The tyrosine kinase receptor TIE-2 was initially identified as a specific endothelial growth factor receptor that mediated several properties of endothelial cells under both physiologcal and pathological conditions. Angiopoietins are the natural ligands of TIE-2 and they induce TIE-2-depending signalling, including survival and apoptosis of endothelial cells, vascular permeability and regulate capillary sprouting. In general, the outcome of tie receptor signalling depends on which vascular bed is involved, and crosstalk between different VEGFs has an important modulating effect on the properties of the ligands. TIE-2 plays an important role in several vascular diseases, known as vascular malformation (e.g. venous malformation). Independent of angiogenesis and its involvement in lymphangiogenesis, TIE-2 maintains a long-term, quiescent population of hematopoietc stems cells in the bone marrow. In a tumor model, a subset of monocytes was found to be positve for TIE-2 and that they have an important function in paracrine support of nascent blood vessels.
During cancer formation and spreading, TIE-2 was found to be overexpressed in tumor vessels. However, outsite the vascular compartment TIE-2 is expressed in several types of cancer including leukemia and in gastric tumors, breast tumors and gliomas. In gliomas, TIE-2 expression in the neoplastic glial cells was significantly associated with progression from a lower to a higher grade where it seems to regulate glioma cell adhesion to extracellular matrix. Together with the reported fact that malignant gliomas epress high levels of Ang1, suggest the existence of an autocrine loop for cell matrix interaction. The possible role of TIE-2 in tumors is more currently under investigation. Because it is expressed in several cellular lineages insite the tumor, the receptor may be an attractive target for cancer therapy.
A natural occuring soluble TIE-2 receptor fragment of 75 kDa (sTIE-2) is generated by shedding. Soluble TIE-2 inhibits angiopoietin-mediated TIE-2 phosphorylation and anti-apoptosis. TIE-2 shedding is mediated

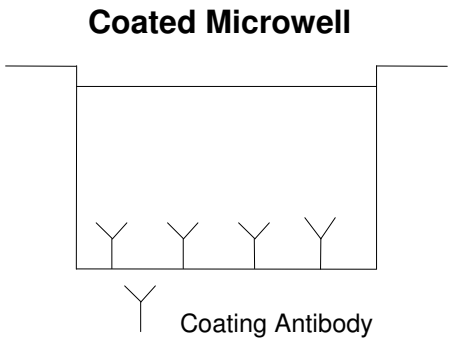
by PI2K/Akt and p38 MAPK. The serum levels of sTIE-2 in patients is in the lower ng/ml range and dependent of the disease. Soluble TIE-2 was also measured in preterm infants including the ROP syndrome (retinopathy of prematurity). Elevated plasma levels in active ROP patients were observed for sTIE-2. In experimental tumor mouse models sTIE-2 can be used for tumor regression and prolonged the tumor-free survival in 80% of the animals.

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

3 Principles of the Test

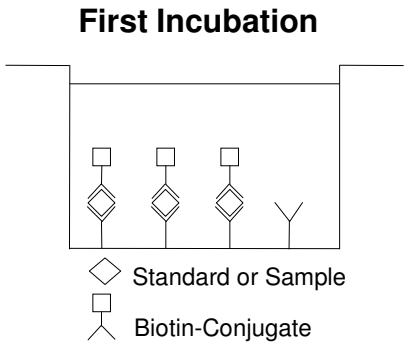
An anti-human TIE-2 coating antibody is adsorbed onto microwells.

Figure 1



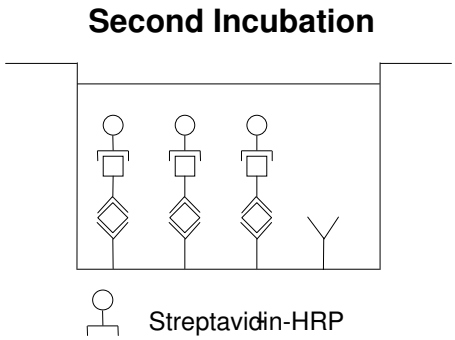
Human TIE-2 present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human TIE-2 antibody is added and binds to human TIE-2 captured by the first antibody.

Figure 2



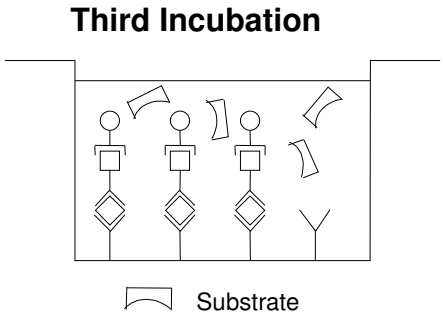
Following incubation unbound biotin-conjugated anti-human TIE-2 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human TIE-2 antibody.

Figure 3



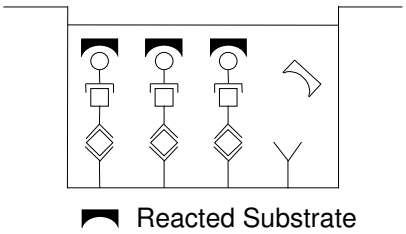
Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Figure 4



A coloured product is formed in proportion to the amount of human TIE-2 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human TIE-2 standard dilutions and human TIE-2 sample concentration determined.

Figure 5



4 Reagents Provided

4.1 Reagents for human TIE-2 ELISA BMS2042 (96 tests)

1 aluminium pouch with a **Microwell Plate coated** with polyclonal antibody to human TIE-2

1 vial (70 µl) **Biotin-Conjugate** anti-human TIE-2 polyclonal antibody

1 vial (150 µl) **Streptavidin-HRP**

2 vials human TIE-2 **Standard** lyophilized, 20.00 ng/ml upon reconstitution

1 vial (12 ml) **Sample Diluent**

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

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

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

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

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

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

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

4 **Adhesive Films**

4.2 Reagents for human TIE-2 ELISA BMS2042TEN
(10x96 tests)

10 aluminium pouches with a **Microwell Plate coated** with polyclonal antibody to human TIE-2

10 vials (70 µl) **Biotin-Conjugate** anti-human TIE-2 polyclonal antibody

10 vials (150 µl) **Streptavidin-HRP**

10 vials human TIE-2 **Standard** lyophilized, 20.00 ng/ml upon reconstitution

10 vials (12 ml) **Sample Diluent**

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

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

10 vials (15 ml) **Substrate Solution** (tetramethyl-benzidine)

10 vials (12 ml) **Stop Solution** (1M Phosphoric acid)

6 vials (0.4 ml) **Blue-Dye**

6 vials (0.4 ml) **Green-Dye**

6 vials (0.4 ml) **Red-Dye**

20 **Adhesive Films**

5 Storage Instructions – ELISA Kit

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.
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, this reagent is not contaminated by the first handling.

6 Specimen Collection and Storage Instructions

Cell culture supernatant, serum and plasma (EDTA, citrate, heparinized), were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Pay attention to a possible **“Hook Effect”** due to high sample concentrations (see chapter 11).

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

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human TIE-2. 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, the frozen sample 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)
- 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 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 statement(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 the conjugate and substrate reagent.

- Exposure to acid inactivates the conjugate.
- 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℃.
- 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

Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure.
If crystals have formed in the **Buffer Concentrates**, warm them gently until they have completely dissolved.

9.1 Wash Buffer (1x)

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** (20x) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 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 Wash Buffer (1x) is stable for 30 days.

Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

9.2 Assay Buffer (1x)

Pour the entire contents (5 ml) 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 to avoid foaming.

Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.

Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

9.3 Biotin-Conjugate

Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (1x) (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

9.4 Streptavidin-HRP

Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (1x) (ml)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

9.5 Human TIE-2 Standard

Reconstitute **human TIE-2 standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 20.00 ng/ml).

After usage remaining standard cannot be stored and has to be discarded.

Standard dilutions can be prepared directly on the microwell plate (see 10.c) or alternatively in tubes (see 9.5.1).

9.5.1 External Standard Dilution

Label 7 tubes, one for each standard point.

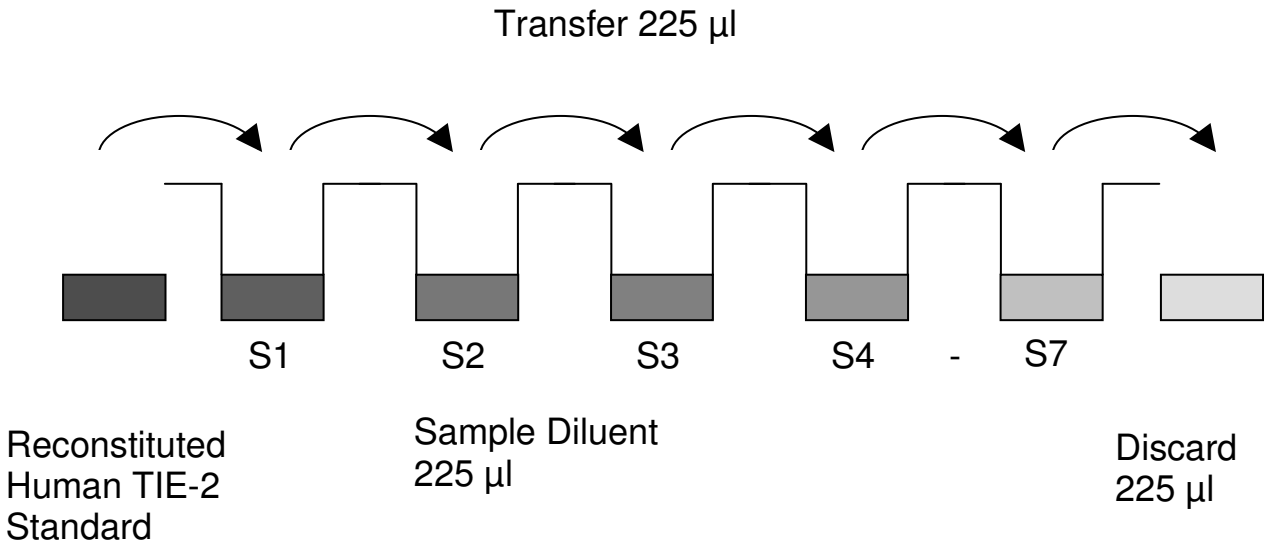
S1, S2, S3, S4, S5, S6, S7

Then prepare 1:2 serial dilutions for the standard curve as follows:
Pipette 225 µl of Sample Diluent into each tube.

Pipette 225 µl of reconstituted standard (concentration of standard = 20.00 ng/ml) into the first tube, labelled S1, and mix (concentration of standard 1 = 10.00 ng/ml).
Pipette 225 µl of this dilution into the second tube, labelled S2, and mix thoroughly before the next transfer.
Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 6).

Sample Diluent serves as blank.

Figure 6



9.6 Addition of Colour-giving Reagents: Blue-Dye, Green-Dye, Red-Dye

In order to help our customers to avoid any mistakes in pipetting the Bender MedSystems ELISAs, Bender MedSystems offers a tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours to each step of the ELISA procedure.

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, Red-Dye***) can be added to the reagents according to the following guidelines:

1. Diluent: Before standard 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 Sample Diluent	20 µl ***Blue-Dye***
12 ml Sample Diluent	48 µl ***Blue-Dye***
50 ml Sample Diluent	200 µl ***Blue-Dye***

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

3 ml Assay Buffer (1x)	30 µl ***Green-Dye***
6 ml Assay Buffer (1x)	60 µl ***Green-Dye***
12 ml Assay Buffer (1x)	120 µl ***Green-Dye***

3. Streptavidin-HRP: Before dilution of the concentrated Streptavidin-HRP, add the ***Red-Dye*** at a dilution of 1:250 (see table below) to the Assay Buffer (1x) used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the instruction booklet: Preparation of Streptavidin-HRP.

6 ml Assay Buffer (1x)	24 µl ***Red-Dye***
12 ml Assay Buffer (1x)	48 µl ***Red-Dye***

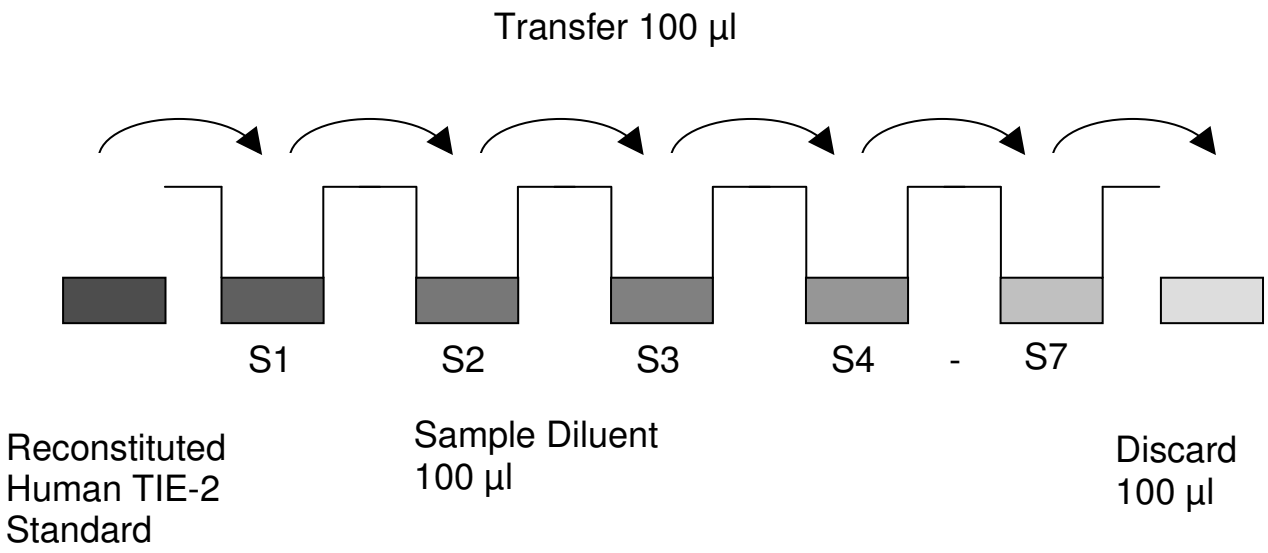
10 Test Protocol

a. 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 from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

b. Wash the microwell strips twice with approximately 400 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells.
After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. **Do not allow wells to dry**.

c. **Standard dilution on the microwell plate** (Alternatively the standard dilution can be prepared in tubes - see 9.5.1):
Add 100 µl of Sample Diluent in duplicate to all **standard wells**. Pipette 100 µl of prepared **standard** (see Preparation of Standard 9.5, concentration = 20.00 ng/ml) in duplicate into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 10.00 ng/ml), and transfer 100 µl to wells B1 and B2, respectively (see Figure 7). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human TIE-2 standard dilutions ranging from 10.00 to 0.16 ng/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 7


Transfer 100 µl
S1
S2
S3
S4
-
S7
Reconstituted
Human TIE-2
Standard
Sample Diluent
100 µl
Discard
100 µl

In case of an **external standard dilution** (see 9.5.1), pipette 100 µl of these standard dilutions (S1 - S7) in the standard wells according to Table 1.

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 (10.00 ng/ml)	Standard 1 (10.00 ng/ml)	Sample 1	Sample 1
B	Standard 2 (5.00 ng/ml)	Standard 2 (5.00 ng/ml)	Sample 2	Sample 2
C	Standard 3 (2.50 ng/ml)	Standard 3 (2.50 ng/ml)	Sample 3	Sample 3
D	Standard 4 (1.25 ng/ml)	Standard 4 (1.25 ng/ml)	Sample 4	Sample 4
E	Standard 5 (0.63 ng/ml)	Standard 5 (0.63 ng/ml)	Sample 5	Sample 5
F	Standard 6 (0.31 ng/ml)	Standard 6 (0.31 ng/ml)	Sample 6	Sample 6
G	Standard 7 (0.16ng/ml)	Standard 7 (0.16 ng/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

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

e. Add 90 µl of **Sample Diluent** to the **sample wells**.

f. Add 10 µl of each **sample** in duplicate to the **sample wells**.

g. Prepare **Biotin-Conjugate** (see Preparation of Biotin-Conjugate 9.3).

h. Add 50 µl of **Biotin-Conjugate** to all wells.

i. Cover with an adhesive film and incubate over night at 4°C, if available on a microplate shaker set at 100 rpm.

j. Prepare **Streptavidin-HRP** (refer to Preparation of Streptavidin-HRP 9.4).

k. Remove adhesive film and empty wells. **Wash** microwell strips 6 times according to point b. of the test protocol. Proceed immediately to the next step.

l. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.

m. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 1 hour, if available on a microplate shaker set at 100 rpm.

n. Remove adhesive film and empty wells. **Wash** microwell strips 6 times according to point b. of the test protocol. Proceed immediately to the next step.

o. Pipette 100 µl of **TMB Substrate Solution** to all wells.

p. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

The colour development on the plate should be monitored and the substrate reaction stopped (see next point 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. Alternatively 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 0.6 – 0.65.

q. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well. 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.

r. 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 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 duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human TIE-2 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human TIE-2 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 TIE-2 concentration.
- **If instructions in this protocol have been followed samples have been diluted 1:10 (10 µl sample + 90 µl Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 10).**
- **Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low human TIE-2 levels (Hook Effect). Such samples require further external predilution according to expected human TIE-2 values with Sample Diluent in order to precisely quantitate the actual human TIE-2 level.**
- It is suggested that each testing facility establishes a control sample of known human TIE-2 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 8. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 8

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

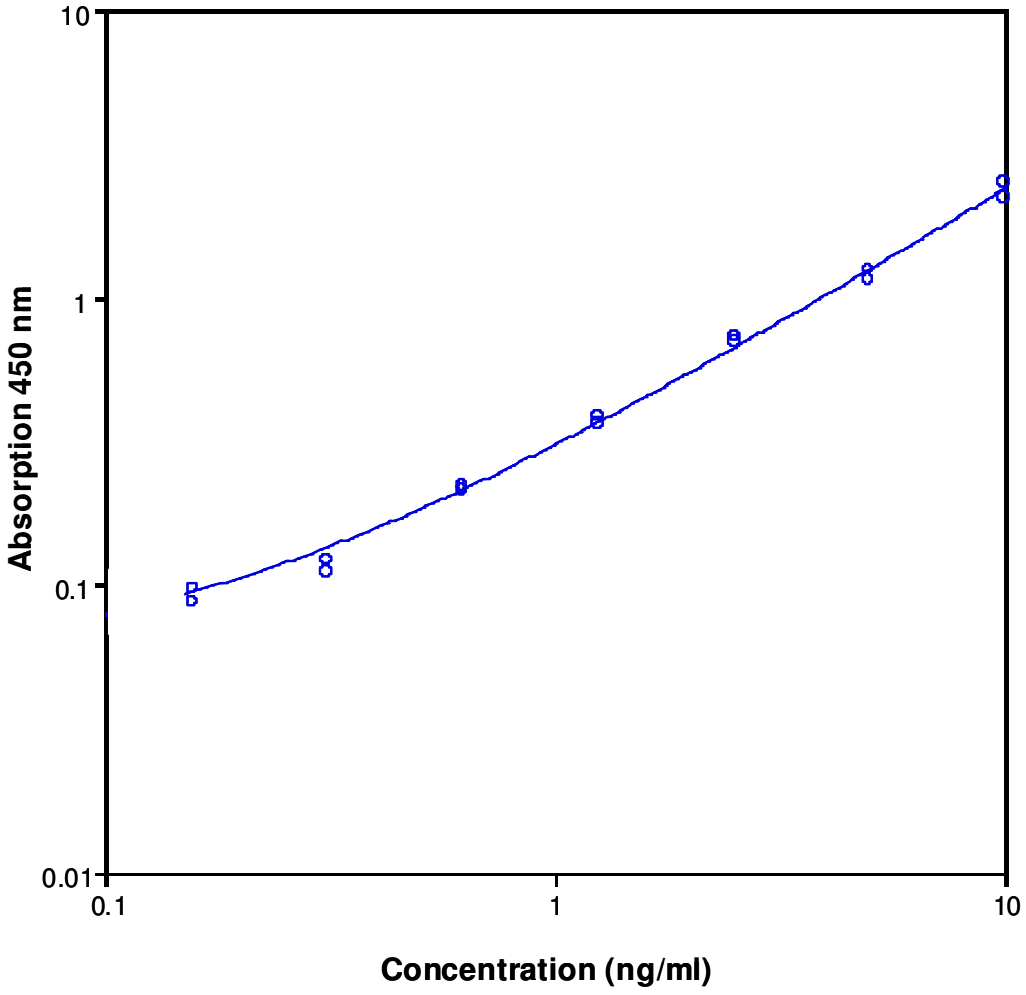


Table 2
Typical data using the human TIE-2 ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human TIE-2 Concentration (ng/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	10.00	2.234 2.528	2.381	6.2
2	5.00	1.143 1.248	1.195	4.4
3	2.50	0.729 0.699	0.714	2.1
4	1.25	0.388 0.364	0.376	3.2
5	0.63	0.221 0.212	0.216	1.9
6	0.31	0.110 0.121	0.115	5.1
7	0.16	0.097 0.087	0.092	5.5
Blank	0.00	0.040 0.043	0.042	2.9

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.

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of human TIE-2 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 0.03 ng/ml (mean of 6 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 6 serum samples containing different concentrations of human TIE-2. 2 standard curves were run on each plate. Data below show the mean human TIE-2 concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 7.1%.

Table 3
The mean human TIE-2 concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Human TIE-2 Concentration (ng/ml)	Coefficient of Variation (%)
1	1	1.8	10.8
	2	2.1	10.9
	3	2.0	9.7
2	1	21.6	1.6
	2	21.5	6.0
	3	23.8	1.9
3	1	11.1	7.3
	2	11.7	6.3
	3	12.8	3.9
4	1	2.1	9.9
	2	2.3	9.3
	3	2.5	5.7
5	1	6.1	8.4
	2	5.7	5.7
	3	6.0	5.6
6	1	29.9	10.9
	2	27.3	5.8
	3	27.9	8.3

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 6 serum samples containing different concentrations of human TIE-2. 2 standard curves were run on each plate. Data below show the mean human TIE-2 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 6.3%.

Table 4
The mean human TIE-2 concentration and the coefficient of variation of each sample

Sample	Mean Human TIE-2 Concentration (ng/ml)	Coefficient of Variation (%)
1	2.0	8.3
2	22.3	5.9
3	11.9	6.9
4	2.3	8.7
5	5.9	3.4
6	28.4	4.9

13.3 Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human TIE-2 into serum. Recoveries were determined in 3 independent experiments with 4 replicates each.
The amount of endogenous human TIE-2 in unspiked serum was subtracted from the spike values.
The recovery ranged from 73% to 102% with an overall mean recovery of 87%.

13.4 Dilution Parallelism

Serum samples with different levels of human TIE-2 were analysed at serial 2 fold dilutions with 4 replicates each.

The recovery ranged from 96.8% to 133.7% with an overall recovery of 108.3% (see Table 5).

Table 5

Sample	Dilution	Expected Human TIE-2 Concentration (ng/ml)	Observed Human TIE-2 Concentration (ng/ml)	Recovery of Expected Human TIE-2 Concentration (%)
1	1:2		45.5	
	1:4	22.7	22.0	96.8
	1:8	11.0	11.5	105.0
	1:16	5.8	6.3	108.6
2	1:2		43.3	
	1:4	21.6	21.4	99.0
	1:8	10.7	10.6	98.7
	1:16	5.3	5.3	100.5
3	1:2		24.2	
	1:4	12.1	15.0	124.4
	1:8	7.5	8.4	111.9
	1:16	4.2	4.6	108.8
4	1:2		27.5	
	1:4	13.8	18.4	133.7
	1:8	9.2	10.4	112.9
	1:16	5.2	5.2	99.7

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 3 times, and the human TIE-2 levels determined. There was no significant loss of human TIE-2 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 at 37°C, and the human TIE-2 level determined after 24 h. There was no significant loss of human TIE-2 immunoreactivity detected during storage under above conditions.

13.6 Specificity

The assay detects both natural and recombinant human TIE-2.
The interference of circulating factors of the immune systeme was evaluated by spiking these proteins at physiologically relevant concentrations into serum.
There was no crossreactivity detected, notably not with TIE-1.

13.7 Expected Values

A panel of sera samples from randomly selected apparently healthy donors (males and females) was tested for human TIE-2.
The detected human TIE-2 levels for sera ranged between 2.9 and 78.4 ng/ml with a mean level of 33.6 ng/ml and a standard deviation of 17.9 ng/ml, for plasma (EDTA) between 5.0 and 54.4 ng/ml with a mean level of 24.1 ng/ml and a standard deviation of 10.0 ng/ml, for plasma (heparinized) between 9.1 and 47.5 ng/ml with a mean level of 27.2 ng/ml and a standard deviation of 9.3 ng/ml and for plasma (citrate) between 2.1 and 45.1 ng/ml with a mean level of 26.4 ng/ml and a standard deviation of 10.8 ng/ml.

14 Ordering Information

For orders please contact:

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Cat.No. BMS2042 human TIE-2 ELISA

Cat.No. BMS2042TEN human TIE-2 ELISA

15 Reagent Preparation Summary

15.1 Wash Buffer (1x)

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

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

15.2 Assay Buffer (1x)

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

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

15.3 Biotin-Conjugate

Make a 1:100 dilution of **Biotin-Conjugate** in Assay Buffer (1x):

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (1x) (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

15.4 Streptavidin-HRP

Make a 1:100 dilution of **Streptavidin-HRP** in Assay Buffer (1x):

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (1x) (ml)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

15.5 Human TIE-2 Standard

Reconstitute lyophilized **human TIE-2 standard** with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

16 Test Protocol Summary

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100 µl Sample Diluent, in duplicate, to all standard wells. Pipette 100 µl prepared standard into the first wells and create standard dilutions by transferring 100 µl from well to well. Discard 100 µl from the last wells.
   Alternatively external standard dilution in tubes (see 9.5.1): Pipette 100 µl of these standard dilutions in the microwell strips.
4. Add 100 µl Sample Diluent, in duplicate, to the blank wells.
5. Add 90 µl Sample Diluent to sample wells.
6. Add 10 µl sample in duplicate, to designated sample wells.
7. Prepare Biotin-Conjugate.
8. Add 50 µl Biotin-Conjugate to all wells.
9. Cover microwell strips and incubate over night at 4°C.
10. Prepare Streptavidin-HRP.
11. Empty and wash microwell strips 6 times with Wash Buffer.
12. Add 100 µl diluted Streptavidin-HRP to all wells.
13. Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C).
14. Empty and wash microwell strips 6 times with Wash Buffer.
15. Add 100 µl of TMB Substrate Solution to all wells.
16. Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
17. Add 100 µl Stop Solution to all wells.
18. Blank microwell reader and measure colour intensity at 450 nm.

Note: If instructions in this protocol have been followed samples have been diluted 1:10 (10 µl sample + 90 µl Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 10).