PRODUCT INFORMATION & MANUAL

Human sHer-2 Instant ELISA

BMS207INST

Enzyme-linked immunosorbent assay for quantitative detection of human sHer-2.

For research use only.

Not for diagnostic or therapeutic procedures.

128 Tests

Human sHer-2 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.bendermedsystems.com

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

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1 Intended Use

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

2 Summary

The human HER-2 (p158 $^{HER-2}$,c-erbB-2, neu) gene encodes a putative transmembrane growth factor receptor (p185 protein) that is closely related to the epidermal growth factor receptor protein. The HER-2 gene product is a 185 kDa-glycoprotein that contains an extracellular ligand-binding domain and intracellular tyrosine kinase activity (9). HER-2 protein staining is observed only in low levels in epithelial cells of most organs in normal human tissues and at slightly higher levels in fetal tissues.

Both HER-2 oncogene amplification and oncoprotein overexpression have been analyzed for potential utility in diagnostic and prognostic tests for: breast (5, 6, 8, 11, 14, 18, 19, 21, 23), ovarian (3, 15, 17, 19, 26), gastric (10, 25), lung (7, 12, 22), and other cancers (1, 4, 5, 16, 20, 24). In these malignancies the HER-2 oncoprotein overexpression is correlated with a poor prognosis.

In 15-40 % of primary breast cancers, amplification of the HER-2 oncogen is found which is highly correlated with overexpression of the encoded 185 kDa protein and seems to play a major role especially during the initiation of ductal carcinomas. HER-2 overexpression is described as independent prognostic factor with greater predictive power than most of the currently used prognostic tools (21) - especially in axillary lymph-node-positive breast cancer patients.

Studies analyzing small series of patients have suggested a prognostic value for HER-2 oncoprotein expression in axillary node negative (ANN) patients. An association between oncoprotein expression and decreased overall survival among ANN patients with good nuclear grade tumors has been demonstrated (13). In addition it has been reported that in low risk patients (estrogen receptor positive, small tumors), HER-2 expression was associated with early recurrence (2). Data demonstrate the large body of evidence implicating HER-2 oncoprotein in the biology and prognosis of breast carcinoma.

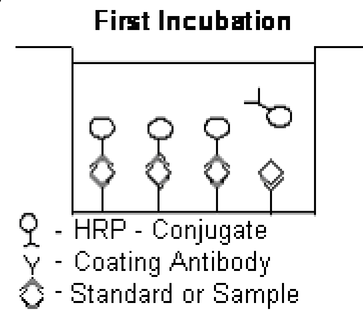
32 % of ovarian carcinomas overexpress the HER-2 oncoprotein. Survival of those patients is significantly worse compared with cases of normal HER-2 protein expression. Additionally, patients whose tumors have high HER-2 protein expression are significantly less likely to have a complete response to primary therapy. Also non-small cell lung cancers which express the HER-2 protein do so at higher levels than those found in normal bronchial epithelium, and expression in adenocarcinoma of the lung is independently associated with diminished survival (7). A correlation between HER-2 expression, and clinical outcome has been also demonstrated for head and neck, salivary gland and placental carcinomas.

HER-2 is useful in identifying cancer cells with increased aggressiveness. Soluble p97-115 $^{HER-2}$ (the soluble circulating fragment of p185 $^{HER-2}$) protein levels in serum can be used as diagnostic tool for monitoring the extent of tumor spread, postoperative relapse and/or metastatic risk for different cancers.

3 Principles of the Test

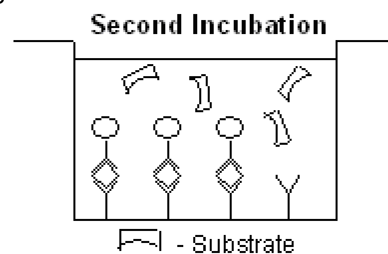
An anti-human sHer-2 coating antibody is adsorbed onto microwells. Human sHer-2 present in the sample or standard binds to antibodies adsorbed to the microwells; an HRP-conjugated anti-human sHer-2 antibody binds to human sHer-2 captured by the first antibody.

Figure 1



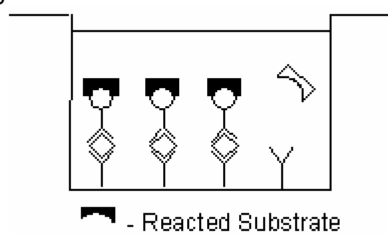
Following incubation unbound enzyme conjugated anti-human sHer-2 is removed during a wash step and substrate solution reactive with HRP is added to the wells.

Figure 2



A coloured product is formed in proportion to the amount of soluble human sHer-2 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 7 human sHer-2 standard dilutions and human sHer-2 sample concentration determined.

Figure 3



4 Reagents Provided

1 aluminium pouch with a **Microwell Plate coated** with monoclonal antibody to human sHer-2, **HRP-Conjugate** (human sHer-2 monoclonal antibody) and Assay Buffer, lyophilized

2 aluminium pouches with a human sHer-2 **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 Films**

5 Storage Instructions

Store ELISA plate and Standard curves or whole kit at -20℃. The plate and the standard curves can also be removed, stored at -20℃, remaining kit reagents can be stored between 2° and 8℃. 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 supernatant and serum were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum from the clot as soon as possible after clotting.

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℃ to avoid loss of bioactive human sHer-2. If samples are to be run within 24 hours, they may be stored at 2° to 8℃ (for sample stability refer to 13).
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
- adjustable multichannel micropipettes (for volumes between 50 µl and 500 µl) 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 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 concentrates should be brought to room temperature and diluted before starting the test procedure. If crystals have formed in **buffer concentrates**, warm them gently until crytals have completely dissolved.

9.1 Wash Buffer

Pour entire contents (25 ml) of the Wash Buffer Concentrate 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. 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 is stable for 30 days.

9.2 Assay Buffer

Pour the entire contents (5ml) of the **Assay Buffer Concentrate** 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℃!**
- **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 so that all the lyophilized pellets remain in the wells.**

a. Prepare your samples before starting with the test procedure. Dilute serum or plasma samples 1:20 with **Assay Buffer** according to the following dilution scheme:
   15 µl sample + 285 µl Assay Buffer

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 and blank should be assayed in duplicate. Remove extra Microwell Strips from holder and store in foil bag with the desiccant provided at -20℃ sealed tig htly. Place Microwell Strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1).

c. Add 50 µl of **distilled water** to the **sample wells**.

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

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 (0.16 ng/ml)	Standard 7 (0.16 ng/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

e. Add 100 µl of each 1:20 prediluted **sample**, in duplicate, to the **designated wells** and mix the contents.

f. Cover with an adhesive film and incubate at room temperature (18ºC to 25ºC) for 3 hours, if available on a microplate shaker at 100 rpm.

g. Remove adhesive film and empty wells. **Wash** the microwell strips 3 times 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, 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 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.

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 th e 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 sHer-2 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.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human sHer-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 sHer-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 sHer-2 concentration.
- ***Samples have been diluted 1:20, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 20).**
- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human sHer-2 levels. Such samples require further external predilution according to expected human sHer-2 values with Assay Buffer (1x) in order to precisely quantitate the actual human sHer-2 level.**
- It is suggested that each testing facility establishes a control sample of known human sHer-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 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 100 µl of the 1:20 prediluted sample. This is a 1:20 dilution.
The remaining 50 µl to give 150 µl are due to the addition of 50 µl conjugate to all wells.
50 µl conjugate correspond to 50 µl reconstitution volume, addition of 100 µl 1:20 prediluted sample (= 1:20 dilution).

Figure 4
Representative standard curve for human sHer-2 Instant ELISA. Human sHer-2 was diluted in serial 2-fold steps in Assay Buffer. Each symbol represents the mean of 3 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.

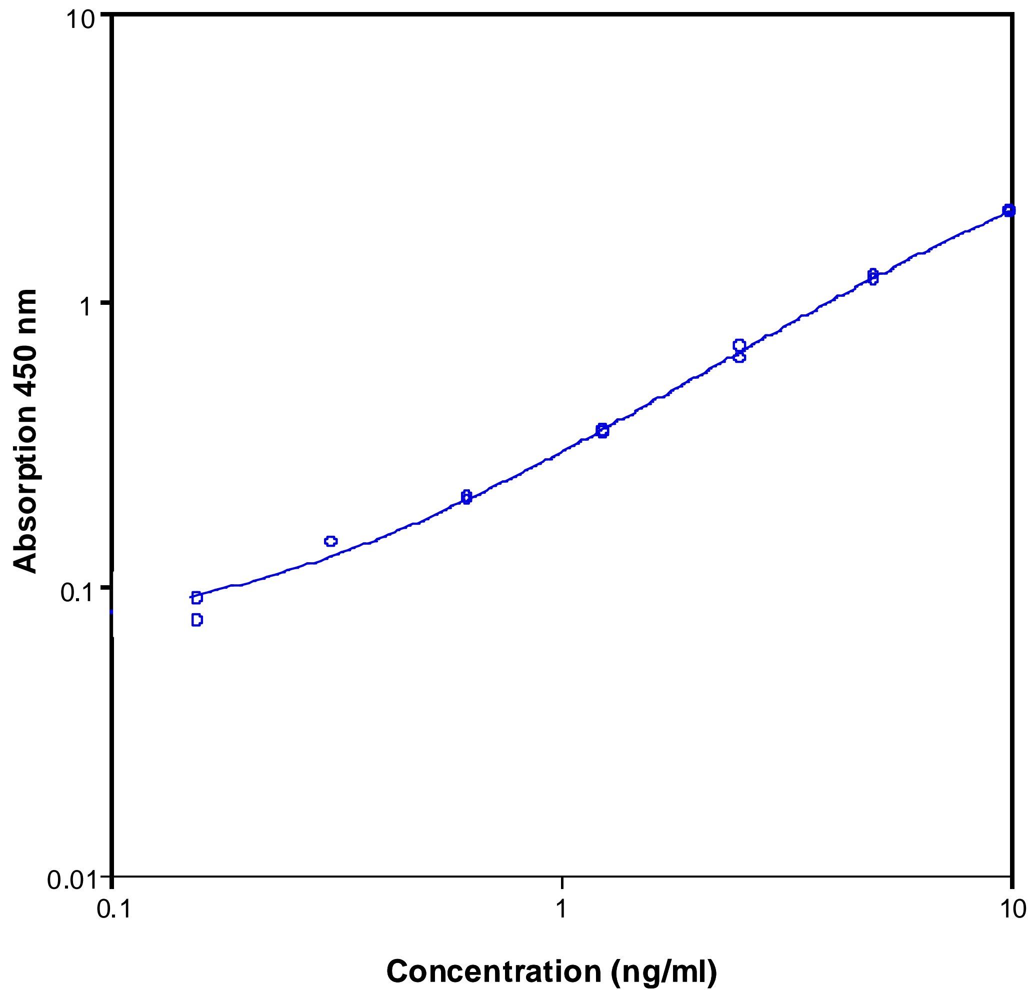


Table 2

Typical data using the human sHer-2 INSTANT ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	human sHer-2 Concentration (ng/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	10.00	2.000 2.052	2.026	3.8
2	5.00	1.175 1.225	1.200	3.6
3	2.50	0.688 0.629	0.659	3.8
4	1.25	0.345 0.353	0.349	6.7
5	0.63	0.207 0.200	0.203	2.0
6	0.31	0.142 0.142	0.142	2.1
7	0.16	0.076 0.091	0.084	8.9
Blank	0.00	0.033 0.046	0.040	

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 sHer-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 0.06 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 sHer-2. 2 standard curves were run on each plate. Data below show the mean human sHer-2 concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 7.2%.

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

Positive Sample	Experiment	human sHer-2 Concentration (ng/ml)	Coefficient of Variation (%)
1	1	165	2
	2	165	3
	3	161	4
2	1	105	5
	2	101	4
	3	97	7
3	1	73	6
	2	66	4
	3	66	6
4	1	14	7
	2	14	11
	3	17	10
5	1	9	10
	2	8	11
	3	10	6
6	1	8	6
	2	6	11
	3	7	14

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 sHer-2. 2 standard curves were run on each plate. Data below show the mean human sHer-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 7.6%.

Table 4

The mean human sHer-2 concentration and the coefficient of variation of each sample

Sample	Mean human sHer-2 Concentration (ng/ml)	Coefficient of Variation (%)
1	164	1.4
2	101	3.9
3	69	5.7
4	15	12.8
5	9	9.8
6	7	12.0
7	164	1.4
8	101	3.9

13.3 Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human sHer-2 into serum. Recoveries were determined in 3 independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments.Average recovery ranged from 90% to 124% with an overall mean recovery of 107%.

13.4 Dilution Parallelism

4 serum samples with different levels of human sHer-2 were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged between 71% and 109% with an overall recovery of 93% (see Table 5).

Table 5

Sample	Dilution	Mean human sHer-2 Concentration (ng/ml)		% Recovery of Exp. Val.
		Expected Value	Observed Value	
1	1:20	--	449	--
	1:40	225	192	86
	1:80	112	99	88
	1:160	56	40	71
2	1:20	--	200	--
	1:40	100	104	104
	1:80	50	46	91
	1:160	25	21	84
3	1:20	--	412	--
	1:40	206	191	93
	1:80	103	103	100
	1:160	52	48	94
4	1:20	--	166	--
	1:40	83	91	109
	1:80	42	38	92
	1:160	21	21	102

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed 5 times, and the human sHer-2 levels determined.
There was no significant loss of human sHer-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 hum an sHer-2 level determined after 24 h.
There was no significant loss of human sHer-2 immunoreactivity detected during storage under above conditions.

13.6 Specificity

The assay detects both natural and recombinant human sHER-2, precisely $p97\text{-}115^{HER\text{-}2}$, the soluble circulating fragment of HER-2 ($p185^{HER\text{-}2}$). To define the specificity of this ELISA several proteins were tested for cross reactivity. There was no cross reactivity determined for IL-8, sICAM-1, sTNF-R, TNF-α, TNF-β, CD8, IL-2, IL-2R, IL-6, IL-6R, IL-10, ELAM-1 and CD44.

13.7 Expected Values

A panel of sera samples from 20 randomly selected apparently healthy donors (males and females) was tested for human sHer-2.
The detected human sHer-2 levels ranged between 3.1 and 30.5 ng /ml with a mean level of 6.8 ng/ml and a standard deviation of 6.4 ng/ml.

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16 Reagent Preparation Summary

16.1 Wash Buffer

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

16.2 Assay Buffer

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

17 Test Protocol Summary

- Predilute sample 1:20 with Assay Buffer
- Place standard strips in position A1/A2 to H1/H2.
- Add 50 µl distilled water to sample wells.
- Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 100 µl 1:20 prediluted sample to designated wells.
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C) if available on a microplate shaker a t 100 rpm.
- Empty and wash microwell strips 3 times with 400 µl Wash Buffer.
- Add 100 µl of TMB Substrate Solution to all wells including blank wells.
- Incubate the microwell strips for about 10 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 1:20, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 20).