# Human HSP90 alpha ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human HSP90 alpha

**Catalog Numbers** BMS2090 or BMS2090TEN

**Pub. No.** MAN0016535 **Rev.** B.0 (31)

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

## Product description

The Human HSP90 alpha ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human HSP90 alpha.

## Summary

HSP90 alpha is a molecular chaperone that belongs to the heat shock protein 90 family predominantly known in helping in the refolding of misfolded proteins or their elimination in case of irreversible damage. The name is simply derived from the molecular weight roughly being 90 Kilo Daltons. There are two existing isoforms, Heat Shock Protein 90 alpha (HSP90 alpha) and Heat Shock Protein 90 beta (HSP90 beta), with 76% homology. HSP90 is a very abundant continuously expressed protein also involved in non-stress related processes such as structural maintenance, cell cycle control and cell signalling. The protein contains three functional domains, the ATP-binding, protein binding and dimerizing domain all essential for the cytoprotecitve properties of HSP90.

## Principles of the test

An anti-human HSP90 alpha coating antibody is adsorbed onto microwells.

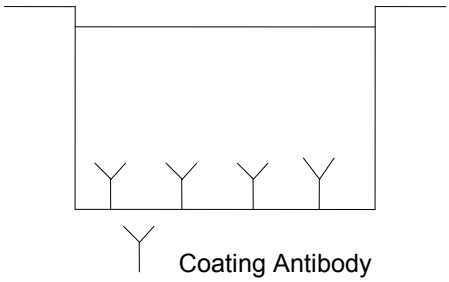


Fig. 1 Coated microwell

Human HSP90 alpha present in the sample or standard binds to antibodies adsorbed to the microwells.

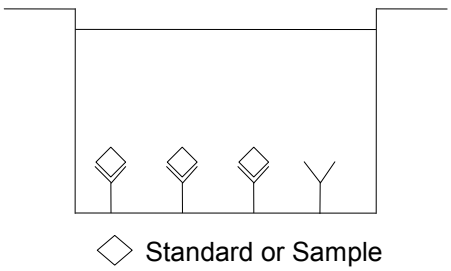


Fig. 2 First incubation

Following incubation unbound biological components are removed during a wash step and a biotin-conjugated anti-human HSP90 alpha antibody is added and binds to human HSP90 alpha captured by the first antibody.

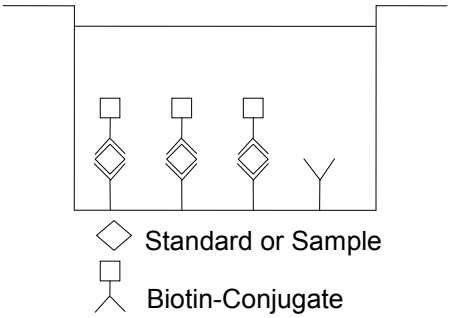


Fig. 3 Second incubation

Following incubation unbound biotin-conjugated anti-human HSP90 alpha antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human HSP90 alpha antibody.

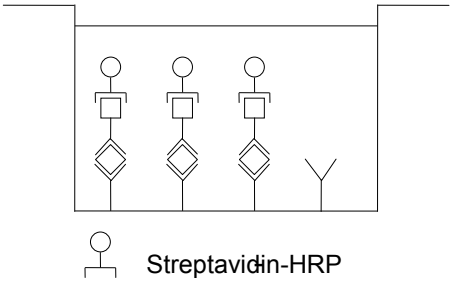


Fig. 4 Third incubation

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

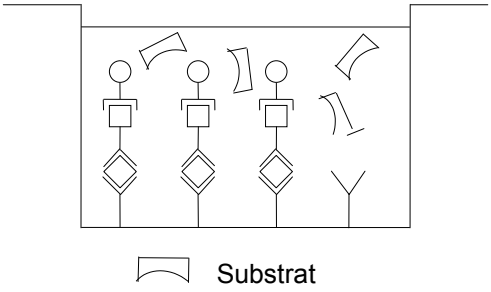


Fig. 5 Fourth incubation

A colored product is formed in proportion to the amount of human HSP90 alpha 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 HSP90 alpha standard dilutions and human HSP90 alpha sample concentration determined.

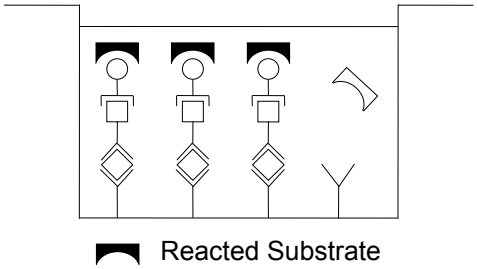


Fig. 6 Stop reaction

## Reagents provided

### Reagents for human HSP90 alpha ELISA BMS2090 (96 tests)

1 aluminum pouch with a Microwell Plate coated with monoclonal antibody to human HSP90 alpha

1 vial (120 µL) Biotin-Conjugate anti-human HSP90 alpha monoclonal antibody

1 vial (150 µL) Streptavidin-HRP

2 vials human HSP90 alpha Standard lyophilized, 20 ng/mL upon reconstitution

2 bottles (15 mL) Sample Diluent

1 vial (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 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 (15 mL) Stop Solution (1M Phosphoric acid)

6 Adhesive Films

### Reagents for human HSP90 alpha ELISA BMS2090TEN (10x96 tests)

10 aluminum pouches with a Microwell Plate coated with monoclonal antibody to human HSP90 alpha

10 vials (120 µL) Biotin-Conjugate anti-human HSP90 alpha monoclonal antibody

10 vials (150 µL) Streptavidin-HRP

10 vials human HSP90 alpha Standard lyophilized, 20 ng/mL upon reconstitution

15 bottles (15 mL) Sample Diluent

3 vials (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 10% BSA)

7 bottles (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)

10 vials (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (100 mL) Stop Solution (1M Phosphoric acid)

30 Adhesive Films

## Storage instructions – ELISA kit

Store kit reagents between 2°C and 8°C. Immediately after use, remaining reagents should be returned to cold storage (2°C 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.

## Sample collection and storage instructions

Cell lysate, serum, and plasma (citrate, heparin) 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.

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

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human HSP90 alpha. If samples are to be run within 24 hours, they may be stored at 2–8°C (for stability refer to “Sample stability“ on page 6).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

## 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 regression analysis

## Precautions for use

- All reagents 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 pipet 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 samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that 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 samples 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.

## Preparation of reagents

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

### 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.
- 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 |

### 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 |

### Biotin-Conjugate

**Note:** 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 | 0.06 | 5.94 |
| 1 - 12 | 0.12 | 11.88 |

### Streptavidin-HRP

**Note:** The Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:200 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 | 0.03 | 5.97 |
| 1 - 12 | 0.06 | 11.94 |

### Human HSP90 alpha standard

1. Reconstitute human HSP90 alpha standard by addition of distilled water.
2. 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 ng/mL).
3. Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.
4. The standard has to be used immediately after reconstitution and cannot be stored.
5. Standard dilutions can be prepared directly on the microwell plate (see "Test protocol" on page 3) or alternatively in tubes (see "External standard dilution" on page 3).

External standard dilution

1. Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7.
2. Prepare 2-fold serial dilutions for the standard curve as follows: Pipette 225 µL of Sample Diluent into each tube.
3. Pipette 225 µL of reconstituted standard (concentration = 20 ng/mL) into the first tube, labeled S1, and mix (concentration of S1 = 10 ng/mL).
4. Pipette 225 µL of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 7).

   Sample diluent serves as blank.

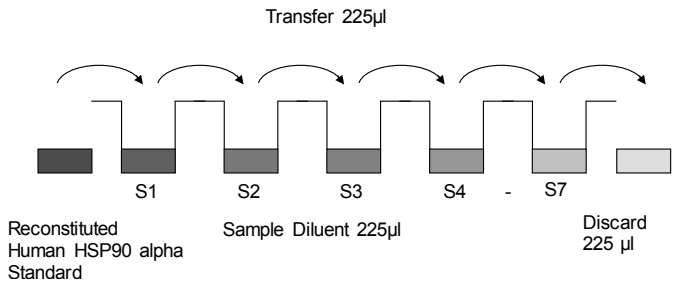


Fig. 7 Dilute standards - tubes

## Test protocol

1. Predilute your samples before starting with the test procedure. Dilute serum and plasma samples 1:25 with Sample Diluent according to the following scheme:

   10 µL Sample + 240 µL Sample Diluent
2. 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.
3. 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.

4. Standard dilution on the microwell plate (alternatively, the standard dilution can be prepared in tubes, see "External standard dilution" on page 3):

   Add 100 µL of Sample Diluent in duplicate to all standard wells. Pipette 100 µL of prepared standard (see "Human HSP90 alpha standard" on page 3, concentration = 20 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 ng/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 8). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human HSP90 alpha standard dilutions, ranging from 10.0 to 0.156 ng/mL. Discard 100 µL of the contents from the last microwells (G1, G2) used.

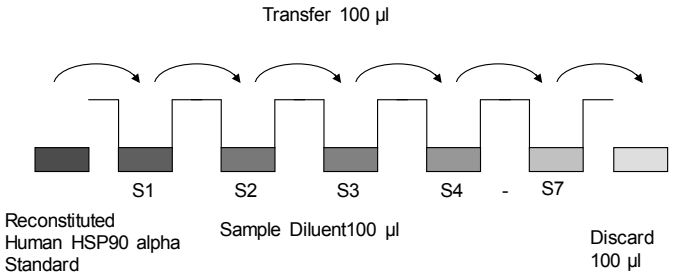


Fig. 8 Dilute standards - microwell plate.

In case of an external standard dilution (see "External standard dilution" on page 3), pipette 100 µL of these standard dilutions (S1 – S7) in the standard wells according to Table 1.

**Table 1** Example of the arrangement of blanks, standards, and samples in the microwell strips.

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| A | Standard 1<br>10.0 ng/mL | Standard 1<br>10.0 ng/mL | Sample 1 | Sample 1 |
| B | Standard 2<br>5.0 ng/mL | Standard 2<br>5.0 ng/mL | Sample 2 | Sample 2 |
| C | Standard 3<br>2.5 ng/mL | Standard 3<br>2.5 ng/mL | Sample 3 | Sample 3 |
| D | Standard 4<br>1.25 ng/mL | Standard 4<br>1.25 ng/mL | Sample 4 | Sample 4 |
| E | Standard 5<br>6.25 ng/mL | Standard 5<br>6.25 ng/mL | Sample 5 | Sample 5 |
| F | Standard 6<br>3.125 ng/mL | Standard 6<br>3.125 ng/mL | Sample 6 | Sample 6 |
| G | Standard 7<br>0.156 ng/mL | Standard 7<br>0.156 ng/mL | Sample 7 | Sample 7 |
| H | Blank | Blank | Sample 8 | Sample 8 |

5. Add 100 µL of Sample Diluent in duplicate to the blank wells.
6. Add 100 µL of each prediluted sample in duplicate to the sample wells.
7. Cover with an adhesive film and incubate at room temperature (18–25°C) for 2 hours, on a microplate shaker set at 400 rpm.
8. Prepare Biotin-Conjugate (see "Biotin-Conjugate" on page 3).
9. Remove adhesive film and empty wells. Wash microwell strips 5 times according to point 3 of the test protocol. Proceed immediately to the next step.
10. Add 100 µL of diluted Biotin-Conjugate to all wells, including the blank wells.
11. Cover with an adhesive film and incubate at room temperature (18–25°C) for 1 hour, on a microplate shaker set at 400 rpm.
12. Prepare Streptavidin-HRP (see "Streptavidin-HRP" on page 3).
13. Remove adhesive film and empty wells. Wash microwell strips 5 times according to point 3 of the test protocol. Proceed immediately to the next step.
14. Add 100 µL of diluted Streptavidin-HRP to all wells, including the blank wells.
15. Cover with an adhesive film and incubate at room temperature (18–25°C) for 30 minutes, on a microplate shaker set at 400 rpm.
16. Remove adhesive film and empty wells. Wash microwell strips 5 times according to point 3 of the test protocol. Proceed immediately to the next step.
17. Pipette 100 µL of TMB Substrate Solution to all wells.
18. Incubate the microwell strips at room temperature (18–25°C) for 30 miutes. Avoid direct exposure to intense light.

    The color 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 color 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 color. Alternatively the color 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.
19. 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.
20. 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:** If instructions of this protocol have been followed, serum and plasma samples have been diluted 1:25 and the concentration read from the standard curve must be multiplied by the dilution factor (x 25).

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

## Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human HSP90 alpha 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 HSP90 alpha 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 HSP90 alpha concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:25 (10 µL sample + 240 µL Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 25).
- It is suggested that each testing facility establishes a control sample of known human HSP90 alpha 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 9.

  **Note:** Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

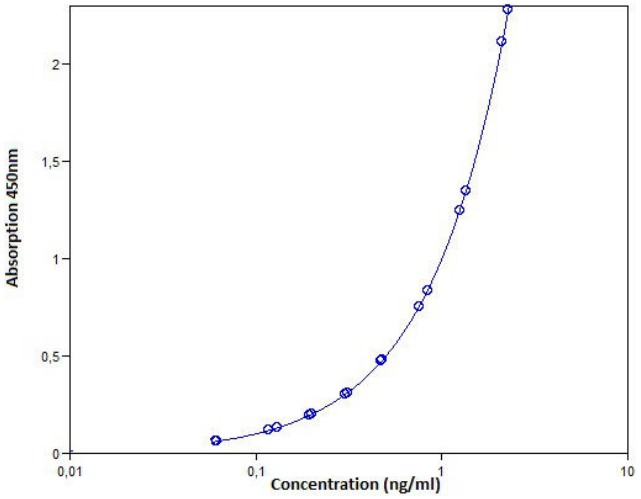


Fig. 9 Representative standard curve for human HSP90 alpha ELISA. Human HSP90 alpha was diluted in serial 2-fold steps in Sample Diluent.

**Table 2** Typical data using the human HSP90 alpha ELISA.

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

| Standard | Human HSP90 alpha Concentration (ng/mL) | O.D. at 450 nm | Mean O.D. at 450 nm | C.V. (%) |
|---|---|---|---|---|
| 1 | 10.0 | 2.115<br>2.282 | 2.141 | 3.2 |
| 2 | 5.0 | 1.352<br>1.250 | 1.224 | 3.8 |
| 3 | 2.5 | 0.752<br>0.839 | 0.724 | 5.4 |
| 4 | 1.25 | 0.473<br>0.481 | 0.420 | 3.9 |
| 5 | 0.63 | 0.303<br>0.311 | 0.234 | 8.2 |
| 6 | 0.31 | 0.195<br>0.200 | 0.130 | 7.9 |
| 7 | 0.16 | 0.130<br>0.118 | 0.062 | 6.8 |
| Blank | 0.0 | 0.062<br>0.061 | | |

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 color intensity. Values measured are still valid.

## 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 analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

## Performance characteristics

### Sensitivity

The limit of detection of human HSP90 alpha 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 4 independent assays).

### Reproducibility

#### Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum and plasma samples containing different concentrations of human HSP90 alpha. Two standard curves were run on each plate. Data below show the mean human HSP90 alpha concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 8.6%.

**Table 3** The mean human HSP90 alpha concentration and the coefficient of variation for each sample.

| Sample | Experiment | Mean human HSP90 alpha concentration (ng/mL) | Coefficient of variation (%) |
|---|---|---|---|
| 1 | 1<br>2<br>3 | 135.1<br>157.7<br>148.7 | 11.2<br>8.5<br>8.4 |
| 2 | 1<br>2<br>3 | 61.6<br>70.9<br>60.9 | 11.7<br>4.2<br>5.2 |
| 3 | 1<br>2<br>3 | 35.9<br>39.9<br>35.0 | 11.0<br>5.5<br>5.6 |
| 4 | 1<br>2<br>3 | 7.1<br>8.6<br>7.1 | 5.7<br>10.3<br>4.8 |
| 5 | 1<br>2<br>3 | 7.8<br>10.1<br>9.5 | 7.6<br>17.9<br>9.0 |
| 6 | 1<br>2<br>3 | 22.7<br>25.1<br>23.8 | 10.5<br>6.1<br>5.6 |
| 7 | 1<br>2<br>3 | 3.9<br>4.0<br>4.9 | 8.5<br>13.4<br>11.2 |
| 8 | 1<br>2<br>3 | 19.1<br>24.2<br>25.3 | 5.9<br>13.1<br>4.7 |

#### 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 and plasma samples containing different concentrations of human HSP90 alpha. Two standard curves were run on each plate. Data below show the mean human HSP90 alpha 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 10.0 %

**Table 4** The mean human HSP90 alpha concentration and the coefficient of variation of each sample.

| Sample | Mean human HSP90 alpha concentration (ng/mL) | Coefficient of variation (%) |
|---|---|---|
| 1 | 147.2 | 7.7 |
| 2 | 64.5 | 8.6 |
| 3 | 36.9 | 7.0 |
| 4 | 7.6 | 11.4 |
| 5 | 9.2 | 13.0 |
| 6 | 23.9 | 5.0 |
| 7 | 4.3 | 11.1 |
| 8 | 22.9 | 7.9 |

### Spike recovery

The spike recovery was evaluated by spiking 3 levels of human HSP90 alpha into serum, plasma (heparin, citrate), and cell lysate samples. Recoveries were determined with 2 replicates each. The amount of endogenous human HSP90 alpha in unspiked samples was subtracted from the spike values.

| Sample matrix | Spike high (%) | | Spike medium (%) | | Spike low (%) | |
|---|---|---|---|---|---|---|
| | Mean | Range | Mean | Range | Mean | Range |
| Serum | 98 | 87–108 | 103 | 98–108 | 87 | 81–99 |
| Plasma (citrate) | 111 | 93–128 | 97 | 90–103 | 92 | 75–111 |
| Plasma (heparin) | 91 | 82–99 | 81 | 78–85 | 68 | 59–78 |
| Cell lysate | 97 | 89–105 | 91 | 89–94 | 83 | 81–85 |

### Dilution parallelism

Serum, plasma (citrate, heparin), and cell lysate samples with different levels of human HSP90 alpha were analyzed at serial 2-fold dilutions with 4 replicates each.

| Sample matrix | Recovery of exp. val. | | |
|---|---|---|---|
| | Dilution | Mean (%) | Range (%) |
| Serum | 1:50 | 111 | 95–122 |
| | 1:100 | 110 | 95–125 |
| | 1:200 | 112 | 98–127 |
| Plasma (citrate) | 1:50 | 105 | 86–112 |
| | 1:100 | 106 | 95–128 |
| | 1:200 | 111 | 95–126 |
| Plasma (heparin) | 1:50 | 101 | 80–116 |
| | 1:100 | 94 | 83–102 |
| | 1:200 | 104 | 95–117 |
| Cell lysate | 1:50 | 85 | 80–90 |
| | 1:100 | 85 | 80–89 |
| | 1:200 | 83 | 77–90 |

### Sample stability

#### Freeze-thaw stability

Aliquots of serum, plasma, cell lysate samples (spiked or unspiked) were stored at -20°C and thawed 3 times, and the human HSP90 alpha levels determined.

There was no significant loss of human HSP90 alpha immunoreactivity detected by freezing and thawing.

#### Storage stability

Aliquots of serum, plasma, and cell lysate samples (spiked or unspiked) were stored at –20°C, 2–8°C, room temperature, and at 37°C, and the human HSP90 alpha level determined after 24 hours. There was no significant loss of human HSP90 alpha immunoreactivity detected during storage under above conditions.

### Specificity

The assay detects both natural and recombinant human HSP90 alpha. The cross-reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human HSP90 alpha positive sample. No cross-reactivity or interference detected.

### Expected values

Panels of 40 serum as well as 16 plasma samples (EDTA, citrate, heparin), from randomly selected healthy donors (males and females) were tested for human HSP90 alpha. For detected human HSP90 alpha levels.

| Sample matrix | Number of samples evaluated | Mean (ng/mL) | Range (ng/mL) | Standard deviation (%) |
|---|---|---|---|---|
| Serum normal | 40 | 37.5 | 10.4–102.8 | 24.8 |
| Plasma (EDTA) | 16 | 4.5 | 1.7–9.2 | 2.4 |
| Plasma (citrate) | 16 | 15.7 | 1.8–28.7 | 7.3 |
| Plasma (heparin) | 16 | 2.4 | 1.5–21.5 | 4.8 |

## Reagent preparation summary

### 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 |

### 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 |

### Biotin-Conjugate

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 | 0.06 | 5.94 |
| 1 - 12 | 0.12 | 11.88 |

### Streptavidin-HRP

Make a 1:200 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 | 0.03 | 5.97 |
| 1 - 12 | 0.06 | 11.94 |

### Human HSP90 alpha standard

Reconstitute human HSP90 alpha standard with distilled water.

(Reconstitution volume is stated on the label of the standard vial.)

## Test protocol summary

1. Predilute your serum and plasma samples with Sample Diluent 1:25
2. Determine the number of microwell strips required.
3. Wash microwell strips twice with Wash Buffer.
4. 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.
5. Add 100 µL of Sample Diluent in duplicate to the blank wells.
6. Add 100 µL of prediluted sample to the sample wells.
7. Cover microwell strips and incubate 2 hours at room temperature (18–25°C), on a microplate shaker.
8. Prepare Biotin-Conjugate.
9. Empty and wash microwell strips 5 times with Wash Buffer.
10. Add 100 µL diluted Biotin-Conjugate to all wells.
11. Cover microwell strips and incubate1 hours at room temperature (18–25°C) on a microplate shaker.
12. Prepare Streptavidin-HRP.
13. Empty and wash microwell strips 5 times with Wash Buffer.
14. Add 100 µL diluted Streptavidin-HRP to all wells.
15. Cover microwell strips and incubate 30 min at room temperature (18–25°C), on a microplate shaker.
16. Empty and wash microwell strips 5 times with Wash Buffer.
17. Add 100 µL of TMB Substrate Solution to all wells.
18. Incubate the microwell strips for about 30 minutes at room temperature (18–25°C)
19. Add 100 µL Stop Solution to all wells.
20. Blank microwell reader and measure color intensity at 450 nm.

**Note:** If instructions in this protocol have been followed, serum and plasma samples have been diluted 1:25 (10 µL sample + 240 µL Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 25).

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**Manufacturer:** Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria