PRODUCT INFORMATION & MANUAL

Human 90K/Mac-2BP Platinum ELISA

BMS234

Enzyme-linked Immunosorbent Assay for quantitative detection of human 90K/Mac-2BP.
For research use only.
Not for diagnostic or therapeutic procedures.

eBioscience®
An Affymetrix Company

Human 90K/Mac-2BP Platinum ELISA

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1. Intended Use

The 90K/Mac-2 BP ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of soluble human 90K/Mac-2 Binding Protein. **The 90K/Mac-2 BP ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2. Summary

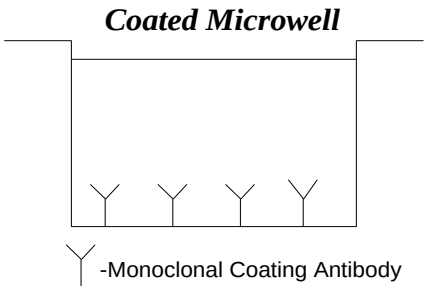
90K/Mac-2 BP is a widely expressed secreted 90 kDa human serum glycoprotein, originally identified in the supernatant of human breast cancer cells. It is identical to Mac-2 BP, the ligand for the secreted lactose-galactose specific S-lectin Mac-2 which is highly expressed by cells of the macrophage-monocyte lineage as well as a variety of other cell types. Although the biological functions and possible roles of 90K/Mac-2 BP are largely unknown, some evidence indicates a role in the immunitary defence mechanism.

Serum levels of 90K/Mac-2 BP have been determined in patients with various forms of neoplasia and in some viral infections. In breast and colorectal cancer and in non-Hodgkin lymphoma, high levels of 90K/Mac-2 BP are correlated with a poor prognosis. In HIV infection, high serum concentrations of 90K/Mac-2 BP may serve as a predictor of faster progression to AIDS, independently of the numbers of CD4+ lymphocytes. In a series of HCV-infected patients, a correlation was found between elevated serum levels of 90K/Mac-2 BP and failure to respond to treatment with α-interferon. The determination of 90K/Mac-2 BP in the serum can therefore be used as a valuable parameter for monitoring the outcome of cancer and viral infections.

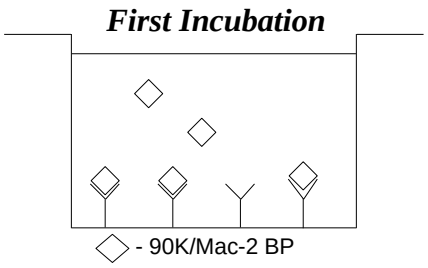
For literature update refer to **www.eBioscience.com**

3. Principles of Test

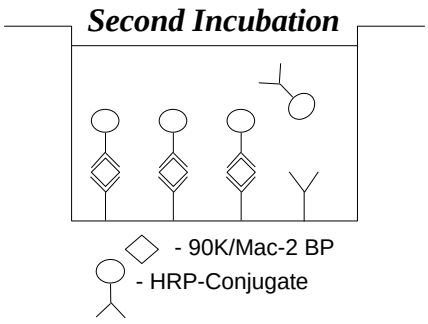
An anti-90K/Mac-2 BP monoclonal coating antibody is adsorbed onto microwells.

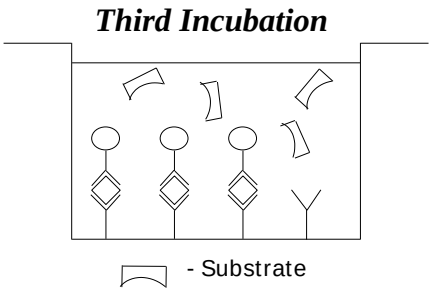


90K/Mac-2 BP present in the sample or standard binds to the antibody adsorbed to the microwells.

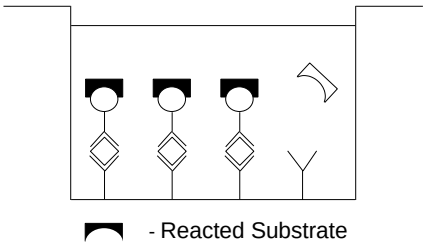


Following incubation a HRP-conjugated monoclonal anti-90K/Mac-2 BP antibody is added and binds to 90K/Mac-2 BP captured by the first antibody. Unbound enzyme conjugated anti-90K/Mac-2 BP is removed during a wash step and substrate solution reactive with HRP is added to the wells.





A coloured product is formed in proportion to the amount of soluble 90K/Mac-2 BP present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from five 90K/Mac-2 BP standards and 90K/Mac-2 BP sample concentration determined.



4. Reagents Provided

1 aluminium pouch with **a Microwell Plate coated with Monoclonal Antibody** (murine) to human 90K/Mac-2 BP

1 bottle (13 ml) **HRP-Conjugate** anti-90K/Mac-2 BP monoclonal (murine) antibody

5 vials (1.0 ml) **90K/Mac-2 BP Standard,** 200ng/ml; 100ng/ml; 50ng/ml;25ng/ml; 12.5ng/ml

2 bottles (50 ml) **Wash Buffer Concentrate** 10x
(PBS with Tween 20)

1 bottle (15 ml) **Sample Diluent** 10x (buffered protein matrix)

1 bottle (12 ml) **Substrate Solution** (tetramethyl-benzidine and hydrogen peroxide, stabilized)

1 vial (16 ml) **Stop Solution** (0.3M Sulfuric Acid)

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

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

2 adhesive **Plate Covers**

5. Storage Instructions – ELISA Kit

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

6. Specimen Collection and Storage Instructions

Cell culture supernatants, serum and plasma (EDTA, citrate, heparin) are able for use in the assay. Remove the serum or plasma from the clot or red cells, respectively, as soon as possible after clotting and separation.

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

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive 90K/Mac-2 BP. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability to 13.6).

Avoid repeated freeze-thaw cycles. Prior to assay, frozen samples should be brought to room temperature slowly and mixed gently and properly diluted with Sample Diluent (1:100 - 1:200 see 10.a).

For sample stability refer to 13.6.

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 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 the conjugate and substrate reagents.

- Exposure to acids will inactivate 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 if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.

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

9. Preparation of Reagents

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 (10x)** into a clean 500 ml graduated cylinder. Bring 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 8°C. Please note that Wash Buffer (1x) is stable for 30 days.

9.2 Sample Diluent (1x)

Mix the contents of the bottle well. Add contents of **Sample Diluent Concentrate (10x)** (15.0 ml) to 135 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Sample Diluent (1x) is stable for 2 weeks at 2° - 8°C.

9.3 Addition of colour-giving reagents: Blue-Dye, Green-Dye

In order to help our customers to avoid any mistakes in pipetting the Platinum ELISAs, eBioscience offers a new 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***) can be added to the reagents according to the following guidelines:

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

5 ml Diluent	20 µl ***Blue-Dye***
12 ml Diluent	48 µl ***Blue-Dye***
50 ml Diluent	200 µl ***Blue-Dye***
60 ml Diluent	240 µl ***Blue-Dye***
100 ml Diluent	400 µl ***Blue-Dye***

2. HRP-Conjugate: Add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the HRP-conjugate.

3 ml HRP-Conjugate	30 µl ***Green-Dye***
6 ml HRP-Conjugate	60 µl ***Green-Dye***
12 ml HRP-Conjugate	120 µl ***Green-Dye***

10. Test Protocol

a. Dilute serum samples 1:100 with Sample Diluent according to the following dilution scheme:

    10 µl Sample + 990 µl Sample Diluent

b. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra **Microwell Strips coated with Monoclonal Antibody** (murine) to human 90K/Mac-2 BP from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

c. Add 100 µl of each **Standard**, in duplicate, to the designated wells (see Figure 1).

Figure 1. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (200.0 ng/ml)	Standard 1 (200.0 ng/ml)	Sample 3	Sample 3
B	Standard 2 (100.0 ng/ml)	Standard 2 (100.0 ng/ml)	Sample 4	Sample 4
C	Standard 3 (50.0 ng/ml)	Standard 3 (50.0 ng/ml)	Sample 5	Sample 5
D	Standard 4 (25.0 ng/ml)	Standard 4 (25.0 ng/ml)	Sample 6	Sample 6
E	Standard 5 (12.5 ng/ml)	Standard 5 (12.5 ng/ml)	Sample 7	Sample 7
F	Blank	Blank	Sample 8	Sample 8
G	Sample 1	Sample 1	Sample 9	Sample 9
H	Sample 2	Sample 2	Sample 10	Sample 10

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

e. Add 100 µl of each 1:100 prediluted **Sample**, in duplicate to the **sample wells**.

f. Cover with a **Plate Cover** and incubate at 37°C for 45 minutes, if available on a rotator set at 400 rpm.

g. Remove Plate Cover and empty wells. Wash the microwell strips four 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 not longer than 15 minutes. Do not allow wells to dry.

h. Add 100 µl of **HRP-Conjugate** to all wells.

i. Cover with a **Plate Cover** and incubate at 37°C for 45 minutes, if available on a rotator set at 400 rpm.

j. Remove **Plate Cover** and empty wells. Wash microwell strips 4 times according to point h. of the test protocol. Proceed immediately to the next step.

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

l. Incubate the microwell strips at room temperature (18° to 25°C) for about 15 minutes, if available on a rotator set at 400 rpm. Avoid direct exposure to intense light.
   The colour development on the plate should be monitored and the substrate reaction stopped (see point m. of this protocol) before positive wells are no longer properly recordable.
   It is recommended to add the stop solution when the highest standard has developed a dark blue colour.
   Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as an OD of 0.9 – 0.95 is reached.

m. 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.

n. 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 90K/Mac-2 BP 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 90K/Mac-2 BP concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating 90K/Mac-2 BP 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 90K/Mac-2 BP concentration.

– **If instructions in this protocol have been followed samples have been diluted 1:100 (10 µl sample + 990 µl Sample Diluent), 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 90K/Mac-2 BP levels. Such samples require further external predilution according to expected 90K/Mac-2 BP values with Sample Diluent in order to precisely quantitate the actual 90K/Mac-2 BP level.**

- It is suggested that each testing facility establishes a control sample of known 90K/Mac-2 BP 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 2. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 2

Representative standard curve for human 90K/Mac-2 BP ELISA. Human 90K/Mac-2 BP 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.

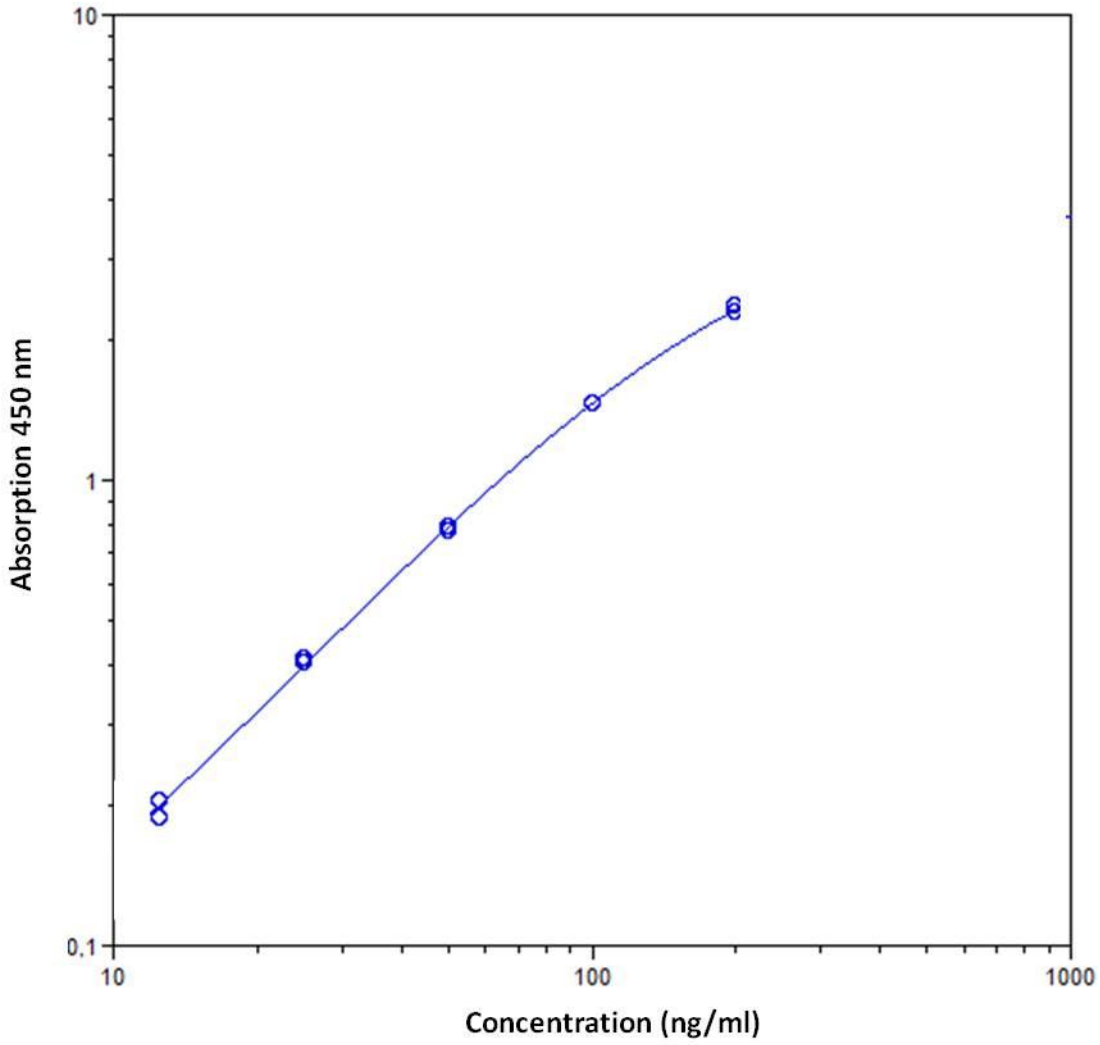


Table 1
Typical data using the human 90K/Mac-2 BP ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human 90K/Mac-2 BP Concentration (ng/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	200.0	2.358 2.286	2.322	1.5
2	100.0	1.465 1.461	1.463	0.2
3	50.0	0.781 0.790	0.785	0.5
4	25.0	0.413 0.403	0.408	1.2
5	12.5	0.188 0.205	0.196	4.4
Blank	0.0	0.025 0.025	0.025	0.0

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 detergent before use.

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

- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibody (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 Diluent.

13. Performance Characteristics

13.1 Sensitivity

The limit of detection for 90K/Mac-2 BP defined as the analyte concentration resulting in an absorption significantly higher than the absorption of the dilution medium (mean plus two standard deviations) was determined to be 0.92 ng/ml (mean of 10 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 5 replicates of 8 samples containing different concentrations of 90K/Mac-2 BP. The overall intra-assay coefficient of variation has been calculated to be 5.5 %.

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by two technicians. Each assay was carried out with 5 replicates of 8 samples containing different concentrations of 90K/Mac-2 BP. The overall inter-assay coefficient of variation has been calculated to be 11.9 %.

13.3 Recovery Studies

Spiked samples were prepared by adding four different levels of recombinant 90K/Mac-2 BP into serum. As shown below, recoveries were ranging from 95% to 139% with an overall mean recovery of 116%.

90K/Mac-2 BP Spike (µg/ml)	Recovery (%) 90K/Mac-2 BP
24.2	139
12.2	108
4.2	121
2.0	95

13.4 Dilution Parallelism

A serum sample was assayed at four two-fold dilutions covering the working range ot the standard curve. In the table below the percent recovery of expected values is listed. Recoveries ranged from 102.4% to 104.3% with an overall mean recovery of 103.7%.

Sample	Dilution	90K/Mac-2 BP Concentration (µg/ml)		
		Expected Value	Observed Value	%Recovery of Exp.Value
1	1:100	20.7	20.7	-
2	1:200	10.4	10.6	102.4
3	1:400	5.2	5.4	104.4
4	1:600	3.5	3.6	104.3

13.5 Expected Values

A panel of 100 sera from healthy blood donors (male and female) was tested for 90K/Mac-2 BP. The detected 90K/Mac-2 BP levels ranged between 1.28 and 16.9 µg/ml with a mean level of 4.2 µg/ml and a standard deviation of 2.9 µg/ml. When the upper limit of the normal range was fixed to 10µg/ml (corresponding to the average plus two standard deviations), 95% of the healthy donors were below this limit.

13.6 Sample Stability

13.6.1 Sample Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked with 90K/Mac-2 BP) were stored at -20°C and thawed several times, and the 90K/Mac-2 BP level determined. There was no significant loss of 90K/Mac-2 BP concentrations between 0 and 5 freeze-thaw cycles.

13.6.2 Sample Storage Stability

Aliquots of a serum sample (unspiked or spiked with 90K/Mac-2 BP) were stored at -20°C, 2-8°C, room temperature and at 37°C and the 90K/Mac-2 BP level determined after 24 hours. There was no significant loss of 90K/Mac-2 BP immunoreactivity during storage under above conditions.

13.7 Comparison of Serum and Plasma

From three individuals, serum as well as EDTA, citrate and heparin plasma obtained at the same time point were evaluated. 90K/Mac-2 BP levels were not significantly different and therefore all these blood preparations are suitable for 90K/Mac-2 BP determinations.

13.8 Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a 90K/Mac-2 BP positive serum. There was no detectable cross reactivity with any of the tested proteins.

14. Ordering Information

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

15. Reagent Preperation Summary

15.1 Wash Buffer (1x)

Add **Wash Buffer Concentrate** 10x (50 ml) to 450 ml distilled water.

15.2 Sample Diluent (1x)

Mix the contents of the bottle well. Add contents of **Sample Diluent Concentrate (10x)** (15.0 ml) to 135 ml distilled or deionized water and mix gently to avoid foaming.

16. Test Protocol Summary

- Dilute samples 1:100 in sample diluent
- Pipette 100 µl **90K/Mac-2 BP Standards** into designated wells
- Add 100 µl **Sample Diluent**, in duplicate, to the blank wells
- Add 100 µl diluted **Sample** to designated wells
- Cover microwell strips and incubate 45 minutes at 37ºC
- Empty and wash microwell strips 4 times with **Wash Buffer**
- Add 100 µl **HRP-Conjugate** to all wells
- Cover microwell strips and incubate 45 minutes at 37°C
- Empty and wash microwell strips 4 times with **Wash Buffer**
- Add 100 µl of **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for about 15 minutes at room temperature (18° to 25°C)
- Add 100 µl **Stop Solution** to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

Note: If instructions in this protocol have been followed samples have been diluted 1:100 (10 µl sample + 990 µl Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 100).