Human s90K/Mac-2BP ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human 90K/Mac-2BP

Catalog Number BMS234

Pub. No. MAN0016608 **Rev.** A.0 (30)

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**.

Intended use

The Human s90K/Mac-2BP ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of soluble human 90K/Mac-2 Binding Protein.

Summary

90K/Mac-2BP 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-2BP, 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-2BP are largely unknown, some evidence indicates a role in the immunitary defence mechanism.

Serum levels of 90K/Mac-2BP 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-2BP are correlated with a poor prognosis. In HIV infection, high serum concentrations of 90K/Mac-2BP 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-2BP and failure to respond to treatment with α-interferon. The determination of 90K/Mac-2BP in the serum can therefore be used as a valuable parameter for monitoring the outcome of cancer and viral infections.

For literature update visit our website.

Principles of test

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

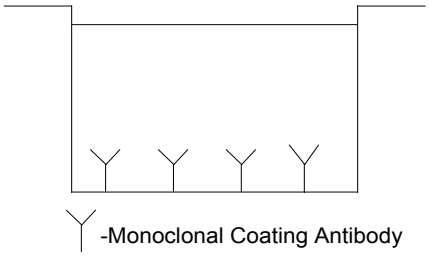

Fig. 1 Coated microwell

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

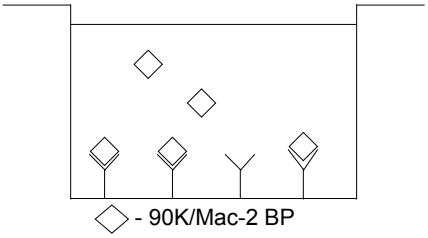

Fig. 2 First incubation

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

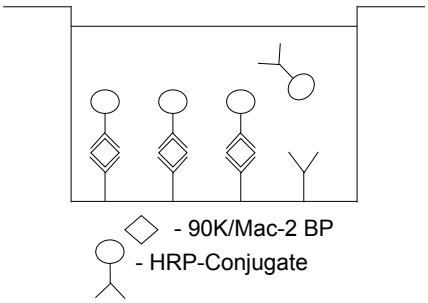

Fig. 3 Second incubation

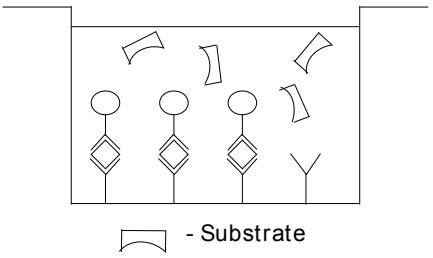

Fig. 4 Third incubation

A colored product is formed in proportion to the amount of soluble 90K/Mac-2BP 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-2BP standards and 90K/Mac-2BP sample concentration determined.

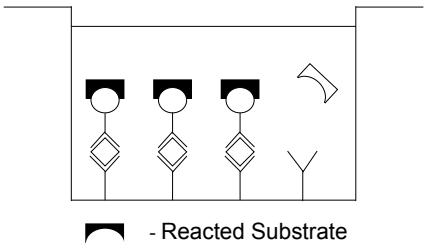

Fig. 5 Stop reaction

Reagents provided

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

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

5 vials (1.0 mL) 90K/Mac-2BP Standard, 200 ng/mL; 100 ng/mL; 50 ng/mL; 25 ng/mL; 12.5 ng/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 (13 mL) Stop Solution (0.16M Sulfuric Acid)

2 adhesive Plate Covers

Storage instructions – ELISA kit

Store kit reagents between 2–8°C. Immediately after use reagents should be returned to cold storage (2–8°C). Expiry of the kit and reagents is stated on labels.

The expiry of the kit components can be guaranteed only 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.

Sample 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 samples.

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive 90K/Mac-2BP. If samples are to be run within 24 hours, they may be stored at 2–8°C (to “Sample stability“ on page 5).

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 “Test protocol“ on page 3).

Refer to “Sample stability“ on page 5.

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

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 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 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 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)

1. 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.
2. Transfer to a clean wash bottle and store at 2–8°C. Note that Wash Buffer (1x) is stable for 30 days.

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 2–8°C. Please note that the Sample Diluent (1x) is stable for 2 weeks at 2–8°C.

Test protocol

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

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

   10 µL Sample + 990 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 coated with Monoclonal Antibody (murine) to human 90K/Mac-2BP from holder and store in foil bag with the desiccant provided at 2–8°C sealed tightly.
3. Add 100 µL of each Standard, in duplicate, to the designated wells (see Table 1).

   Table 1 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

4. Add 100 µL of Sample Diluent, in duplicate, to the blank wells.
5. Add 100 µL of each 1:100 prediluted Sample, in duplicate to the sample wells.
6. Cover with a Plate Cover and incubate at 37°C for 45 minutes, if available on a rotator set at 400 rpm.
7. 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.
8. Add 100 µL of HRP-Conjugate to all wells.
9. Cover with a Plate Cover and incubate at 37°C for 45 minutes, if available on a rotator set at 400 rpm.
10. Remove Plate Cover and empty wells. Wash microwell strips 4 times according to step 7 of the test protocol. Proceed immediately to the next step.
11. Pipette 100 µL of TMB Substrate Solution to all wells, including the blank wells.
12. Incubate the microwell strips at room temperature (18–25°C) for about 15 minutes, if available on a rotator set at 400 rpm. Avoid direct exposure to intense light.

    The color development on the plate should be monitored and the substrate reaction stopped (see point 13. 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 color.

    Alternatively the color 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.
13. 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.
14. 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-2BP standards.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the 90K/Mac-2BP concentration on the abscissa. Draw a best fit curve through the points of the graph.
- To determine the concentration of circulating 90K/Mac-2BP 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-2BP concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:100 (10 µL sample + 990 µL Sample Diluent) and 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-2BP levels. Such samples require further external predilution according to expected 90K/Mac-2BP values with Sample Diluent in order to precisely quantitate the actual 90K/Mac-2BP level.
- It is suggested that each testing facility establishes a control sample of known 90K/Mac-2BP 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 6.

  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.

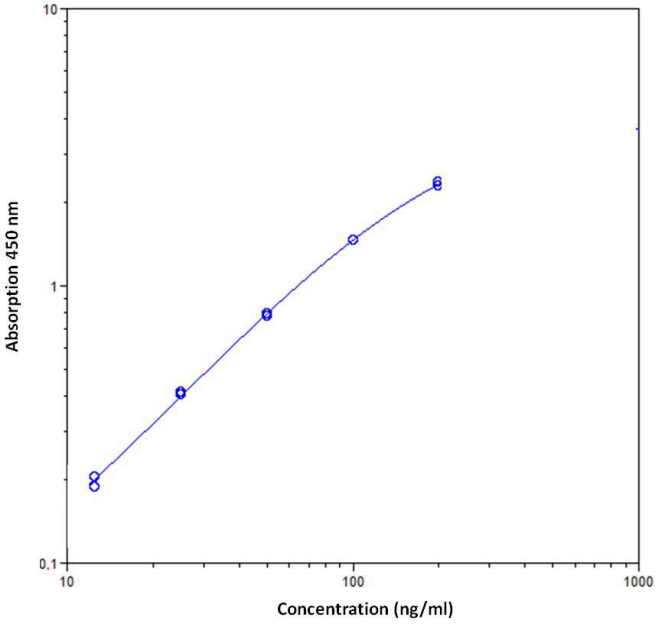

Fig. 6 Representative standard curve for human 90K/Mac-2BP ELISA. Human 90K/Mac-2BP was diluted in serial 2-fold steps in Sample Diluent.

Table 2 Typical data using the human 90K/Mac-2BP ELISA (measuring wavelength of 450 nm and reference wavelength of 620 nm).

Standard	Human 90K/Mac-2BP 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 color intensity. Values measured are still valid.

Limitations

- Because 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.

Performance characteristics

Sensitivity

The limit of detection for 90K/Mac-2BP 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).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 5 replicates of 8 samples containing different concentrations of 90K/Mac-2BP. The overall intra-assay coefficient of variation has been calculated to be 5.5 %.

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments by 2 technicians. Each assay was carried out with 5 replicates of 8 samples containing different concentrations of 90K/Mac-2BP. The overall inter-assay coefficient of variation has been calculated to be 11.9%.

Recovery studies

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

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

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-2BP	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

Expected values

A panel of 100 sera from healthy blood donors (male and female) was tested for 90K/Mac-2BP. The detected 90K/Mac-2BP 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 10 µg/mL (corresponding to the average plus two standard deviations), 95% of the healthy donors were below this limit.

Sample stability

Sample freeze-thaw stability

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

Sample storage stability

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

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-2BP levels were not significantly different and therefore all these blood preparations are suitable for 90K/Mac-2BP determinations.

Specificity

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

Reagent preperation summary

Wash buffer (1x)

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

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.

Test protocol summary

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

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

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria