Rat IL-6 ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of rat IL-6

Catalog Numbers BMS625 or BMS625TWO or BMS625TEN

Pub. No. MAN0016898 **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 Rat IL-6 ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of rat IL-6.

Summary

Interleukin-6 (IL-6) is a multifunctional cytokine that regulates immune responses, acute phase reactions, and hematopoiesis, and may play a central role in host defense mechanisms.

IL-6 is usually not produced constitutively by normal cells, but its expression is readily induced by a variety of cytokines, lipopolysaccharide, or viral infections.

IL-6 is a pleiotropic cytokine produced by a variety of cells. It acts on a wide range of tissues, exerting growth-induction, growth-inhibition, and differentiation, respectively, depending on the nature of the target cells.

IL-6 is involved in:

- The induction of B-cell differentiation
- The induction of acute phase proteins in liver cells
- Growth promotion of myeloma/plasmacytoma/hybridoma cells
- Induction of IL-2 and IL-2 receptor expression
- Proliferation and differentiation of T cells
- Inhibition of cell growth of certain myeloid leukemic cell lines and induction of their differentiation to macrophages
- Enhancement of IL-3-induced multipotential colony cell formation in hematopoietic stem cells and induction of maturation of megakaryocytes as a thrombopoietic factor
- Induction of mesangial cell growth
- Induction of neural differentiation of PC 12 cells
- Induction of keratinocyte growth

The abnormal production of IL-6 was first suggested to be related to polyclonal B-cell activation with auto-antibody production in patients with cardiac myxoma. Since then, IL-6 has been suggested to be involved in the pathogenesis of a variety of diseases. Measurement of IL-6 levels in serum and other body fluids thus provides more detailed insights into various pathological situations.

For literature update visit our website.

Principles of the test

An anti-rat IL-6 coating antibody is adsorbed onto microwells.

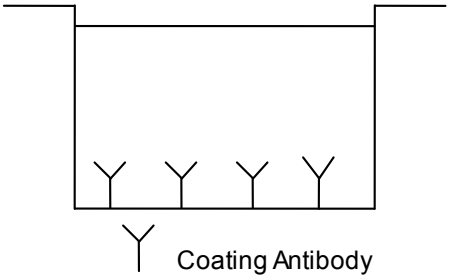

Fig. 1 Coated microwell.

Rat IL-6 present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-rat IL-6 antibody is added and binds to rat IL-6 captured by the first antibody.

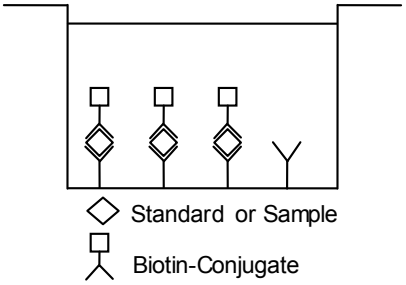

Fig. 2 First incubation.

Following incubation unbound biotin-conjugated anti-rat IL-6 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-rat IL-6 antibody.

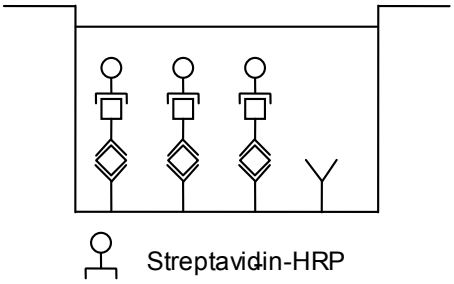

Fig. 3 Second incubation.

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

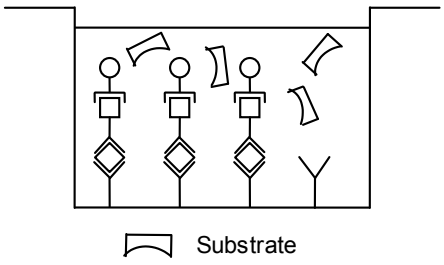

Fig. 4 Third incubation.

A colored product is formed in proportion to the amount of rat IL-6 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 rat IL-6 standard dilutions and rat IL-6 sample concentration determined.

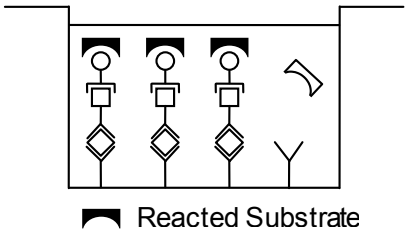

Fig. 5 Stop reaction.

Reagents provided

Reagents for rat IL-6 ELISA BMS625 (96 tests)

1 aluminum pouch with a Microwell Plate coated with monoclonal antibody to rat IL-6

1 vial (70 µL) Biotin-Conjugate anti-rat IL-6 monoclonal antibody

1 vial (150 µL) Streptavidin-HRP

2 vials rat IL-6 Standard lyophilized, 4 ng/mL upon reconstitution

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)

4 Adhesive Films

Reagents for rat IL-6 ELISA BMS625TWO (2x96 tests)

2 aluminum pouches with a Microwell Plate coated with monoclonal antibody to rat IL-6

2 vials (70 µL) Biotin-Conjugate anti-rat IL-6 monoclonal antibody

2 vials (150 µL) Streptavidin-HRP

4 vials rat IL-6 Standard lyophilized, 4 ng/mL upon reconstitution

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

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

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

2 vials (15 mL) Stop Solution (1M Phosphoric acid)

8 Adhesive Films

Reagents for rat IL-6 ELISA BMS625TEN (10x96 tests)

10 aluminum pouches with a Microwell Plate coated with monoclonal antibody to rat IL-6

10 vials (70 µL) Biotin-Conjugate anti-rat IL-6 monoclonal antibody

10 vials (150 µL) Streptavidin-HRP

10 vials rat IL-6 Standard lyophilized, 4 ng/mL upon reconstitution

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

6 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)

20 Adhesive Films

Storage instructions – ELISA kit

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

Samples collection and storage instructions

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 samples.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive rat IL-6. If samples are to be run within 24 hours, they may be stored at 2–8°C (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 1,000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipettes with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, and cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

Precautions for use

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or 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)

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (20x) into a clean 1,000 mL graduated cylinder. Bring to final volume of 1,000 mL with glass-distilled or deionized water.
2. Mix gently to avoid foaming.
3. Transfer to a clean wash bottle and store at 2° to 25°C. Note that Wash Buffer (1x) is stable for 30 days.
4. 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)

1. 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.
2. Store at 2–8°C. Please note that the Assay Buffer (1x) is stable for 30 days.
3. 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	0.03	2.97
1–12	0.06	5.94

Streptavidin-HRP

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

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

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

Rat IL-6 standard

1. Reconstitute rat IL-6 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 = 4 ng/mL). Allow the reconstituted standard to sit for 10–30 minutes. Mix well prior to making dilutions.
3. After usage remaining standard cannot be stored and has to be discarded.
4. Standard dilutions can be prepared directly on the microwell plate 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 1:2 serial dilutions for the standard curve as follows: Pipette 225 µL of Assay Buffer (1x) into each tube.
3. Pipette 225 µL of reconstituted standard (concentration = 4 ng/mL) into the first tube, labeled S1, and mix (concentration of standard 1 = 2 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 6).

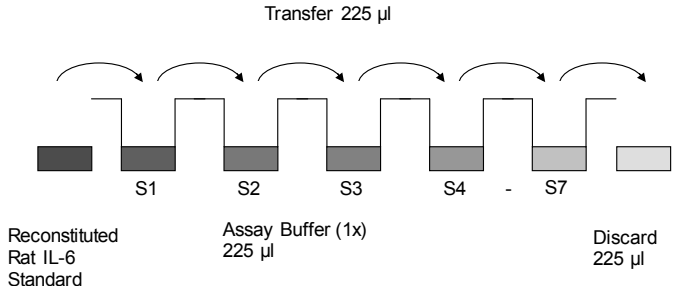

Fig. 6 Dilute standards - tubes.

Assay Buffer (1x) serves as blank.

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. 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 the holder and store in a foil bag with the desiccant provided at 2–8°C sealed tightly.
2. 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.
3. 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 Assay Buffer (1x) in duplicate to all standard wells. Pipette 100 µL of prepared standard (see "Rat IL-6 standard" on page 3, concentration = 4,000.0 pg/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 = 2,000.0 pg/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 7). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of rat IL-6 standard dilutions ranging from 2,000.0 to 31 pg/mL. Discard 100 µL of the contents from the last microwells (G1/G2) used.

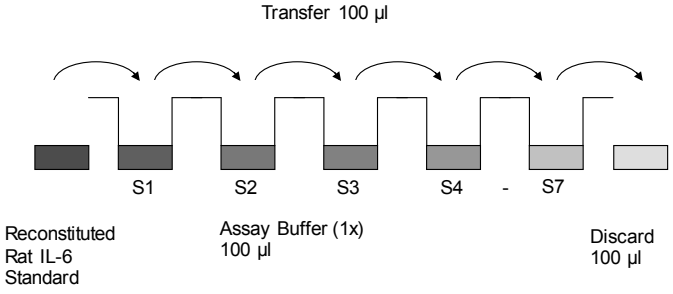

Fig. 7 Dilute standards - microwell plate.

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 2,000.0 pg/mL	Standard 1 2,000.0 pg/mL	Sample 1	Sample 1
B	Standard 2 1,000.0 pg/mL	Standard 2 1,000.0 pg/mL	Sample 2	Sample 2
C	Standard 3 500.0 pg/mL	Standard 3 500.0 pg/mL	Sample 3	Sample 3
D	Standard 4 250.0 pg/mL	Standard 4 250.0 pg/mL	Sample 4	Sample 4
E	Standard 5 125.0 pg/mL	Standard 5 125.0 pg/mL	Sample 5	Sample 5
F	Standard 6 62.5 pg/mL	Standard 6 62.5 pg/mL	Sample 6	Sample 6
G	Standard 7 31.3 pg/mL	Standard 7 31.3 pg/mL	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

4. Add 100 µL of Assay Buffer (1x) in duplicate to the blank wells.
5. Add 50 µL of Assay Buffer (1x) to the sample wells.
6. Add 50 µL of each sample in duplicate to the sample wells.
7. Prepare Biotin-Conjugate (see "Biotin-Conjugate" on page 3).
8. Add 50 µL of Biotin-Conjugate to all wells.
9. Cover with an adhesive film and incubate at room temperature (18–25°C) for 2 hours, if available on a microplate shaker set at 400 rpm.
10. Prepare Streptavidin-HRP (see "Streptavidin-HRP" on page 3).
11. Remove adhesive film and empty wells. Wash microwell strips 6 times according to step 2. Proceed immediately to the next step.
12. Add 100 µL of diluted Streptavidin-HRP to all wells, including the blank wells.
13. Cover with an adhesive film and incubate at room temperature (18–25°C) for 1 hour, if available on a microplate shaker set at 400 rpm.
14. Remove adhesive film and empty wells. Wash microwell strips 6 times according to point b. of the test protocol. Proceed immediately to the next step.
15. Pipette 100 µL of TMB Substrate Solution to all wells.
16. Incubate the microwell strips at room temperature (18–25°C) for about 10 minutes. Avoid direct exposure to intense light.

    The color development on the plate should be monitored and the substrate reaction stopped (see next step) 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.
17. 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.
18. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610–650 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.

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 rat IL-6 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 rat IL-6 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 rat IL-6 concentration.

  If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µL sample + 50 µL Assay Buffer (1x)) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

  Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low rat IL-6 levels. Such samples require further external predilution according to expected rat IL-6 values with Assay Buffer (1x) in order to precisely quantitate the actual rat IL-6 level.
- It is suggested that each testing facility establishe a control sample of known rat IL-6 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 8. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

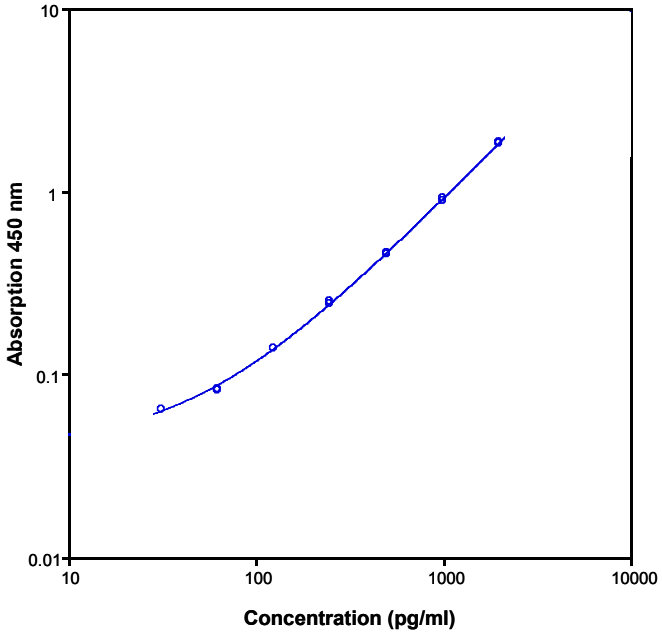

Fig. 8 Representative standard curve for Rat IL-6 ELISA Kit. Rat IL-6 was diluted in serial 2-fold steps in Assay Buffer (1x). Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Table 2 Typical data using the Rat IL-6 ELISA Kit (measuring wavelength is 450 nm, reference wavelength is 620 nm)

Standard	Rat IL-6 concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	2,000.0	1.863 1.815	1.839	1.8
2	1,000.0	0.896 0.923	0.910	2.1
3	500.0	0.459 0.455	0.457	0.6
4	250.0	0.243 0.250	0.247	2.0
5	125.0	0.139 0.138	0.139	0.5
6	62.5	0.082 0.083	0.083	0.9
7	31.3	0.064 0.064	0.064	0.0
Blank	0.0	0.038 0.037	0.038	1.3

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 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.

Performance characteristics

Sensitivity

The limit of detection of rat IL-6 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 12 pg/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of rat serum samples containing different concentrations of rat IL-6. Two standard curves were run on each plate. The calculated overall intra-assay coefficient of variation was <5%.

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of rat serum samples containing different concentrations of rat IL-6. Two standard curves were run on each plate. The calculated overall inter-assay coefficient of variation was <10%.

Dilution parallelism

Rat serum samples with different levels of rat IL-6 were analyzed at serial 2-fold dilutions with 4 replicates each. The overall mean recovery was 97%.

Sample stability

Freeze-thaw stability

Aliquots of spiked serum samples were stored at –20°C and thawed 5 times, and the rat IL-6 levels determined. There was no significant loss of rat IL-6 immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of spiked serum samples were stored at –20°C, 2–8°C, room temperature, and at 37°C, and the rat IL-6 level determined after 24 hours. There was no significant loss of rat IL-6 immunoreactivity detected during storage at –20°C, 2–8°C, and room temperature. A significant loss of rat IL-6 immunoreactivity was detected during storage at 37°C after 24 hours.

Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a rat IL-6 positive serum. There was no cross-reactivity detected.

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 Biotin-Conjugate in Assay Buffer (1x):

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

Streptavidin-HRP

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

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

Rat IL-6 standard

Reconstitute lyophilized rat IL-6 standard with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

Test protocol summary

If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µL sample + 50 µL Assay Buffer (1x)) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100 µL Assay Buffer (1x), in duplicate, to all standard wells. Pipette 100 µL prepared standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells.

   Alternatively, external standard dilution in tubes (see "External standard dilution" on page 3): Pipette 100 µL of these standard dilutions in the microwell strips.
4. Add 100 µL Assay Buffer (1x), in duplicate, to the blank wells.
5. Add 50 µL Assay Buffer (1x) to sample wells.
6. Add 50 µL sample in duplicate, to designated sample wells.
7. Prepare Biotin-Conjugate.
8. Add 50 µL Biotin-Conjugate to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18–25°C).
10. Prepare Streptavidin-HRP.
11. Empty and wash microwell strips 6 times with Wash Buffer.
12. Add 100 µL diluted Streptavidin-HRP to all wells.
13. Cover microwell strips and incubate 1 hour at room temperature (18–25°C).
14. Empty and wash microwell strips 6 times with Wash Buffer.
15. Add 100 µL of TMB Substrate Solution to all wells.
16. Incubate the microwell strips for about 10 minutes at room temperature (18–25°C).
17. Add 100 µL Stop Solution to all wells.
18. Blank microwell reader and measure color intensity at 450 nm.

Customer and technical support

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  - Safety Data Sheets (SDSs; also known as MSDSs)

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

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