

PRODUCT INFORMATION AND MANUAL

mouse IL-10 Instant ELISA

BMS614INST

Enzyme-linked immunosorbent assay for quantitative detection of mouse Interleukin-10.

For research use only.

Not for diagnostic or therapeutic procedures.

96 Tests

***mouse IL-10
BMS614INST***



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1. INTENDED USE

The mIL-10 Instant ELISA is an enzyme-linked immunosorbent assay for quantitative detection of murine Interleukin-10 in cell culture supernatants, murine serum, plasma or other body fluids. **The mIL-10 Instant ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2. SUMMARY

Interleukin-10 is a pleiotropic cytokine playing an important role as a regulator of lymphoid and myeloid cell function. Due to the ability of IL-10 to block cytokine synthesis and several accessory cell functions of macrophages, T-cells and NK cells. In addition, IL-10 participates in regulating proliferation and differentiation of B-cells, mast cells and thymocytes (9).

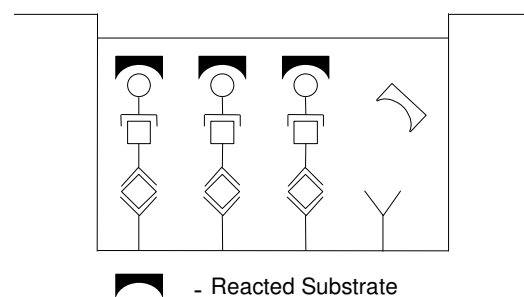
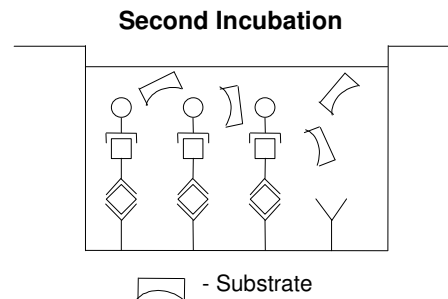
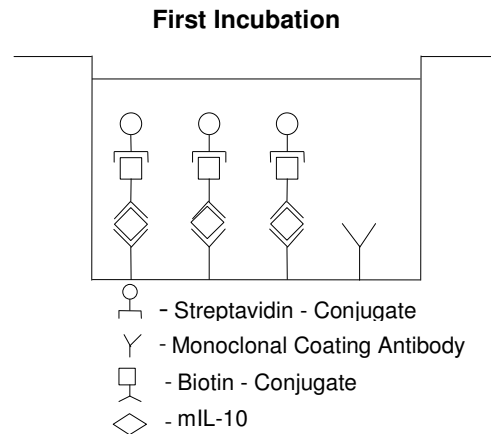
The murine IL-10 exhibits strong DNA and aminoacid sequence homology to the human IL-10 and an open reading frame in the Epstein-Barr virus genome, BCRF1 (1, 8, 15) which shares many of the cellular cytokine's biological activities and may therefore play a role in the host-virus interaction.

3. PRINCIPLES OF THE TEST

An anti-mIL-10 monoclonal coating antibody is adsorbed onto microwells. mIL-10 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated monoclonal anti-mIL-10 antibody binds to mIL-10 captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated anti-mIL-10.

Following incubation unbound biotin conjugated anti-mIL-10 and Streptavidin-HRP 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 mIL-10 present in the sample. The reaction is terminated by addition of acid and adsorbance is measured at 450 nm. A standard curve is prepared from seven mIL-10 standard dilutions and mIL-10 sample concentration determined.



4. REAGENTS PROVIDED

- 1 aluminium pouch with a **Microwell Plate coated with Monoclonal Antibody** (rat) to murine IL-10, **Biotin-Conjugate** (anti-mIL-10 monoclonal antibody), **Streptavidin-HRP** and Sample Diluent, lyophilized
- 2 aluminium pouches with a **mouse IL-10 Standard curve (coloured)**
- 1 bottle (25 ml) **Wash Buffer Concentrate 20x** (PBS with 1% Tween 20)¹⁾
- 1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)
- 1 vial (12 ml) **Sample Diluent** (Use in case an external predilution of the samples is needed)
- 1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)
- 2 adhesive **Plate Covers**

¹⁾ reagents contain preservative

5. STORAGE INSTRUCTIONS

Store ELISA plate or whole kit at -20°C. The plate can also be removed, stored at -20°C, remaining kit reagents stored between 2° and 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

Cell culture supernatants, murine serum, plasma or other biological samples will be suitable for use in the assay. Remove serum 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.

Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive mIL-10. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

For stability and suitability of samples refer to respective chapter.

7. MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes
- 10 μ l to 1,000 μ l adjustable single channel micropipettes with disposable tips
- 50 μ l to 300 μ l 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

- 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.
- 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

Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (25 ml) of the **Wash Buffer Concentrate** into a clean 500 ml graduated cylinder. Bring final volume to 500 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days.

10. TEST PROTOCOLL

- **Use plate immediately after removal from -20 °C!**
 - **Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water!**
 - **Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results**
 - **Perform the washing step with at least 400µl of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results**
Allow the washing buffer to sit in the wells for a few seconds before aspiration
 - **Remove covers of the standard strips carefully in order that all the lyophilised pellets remain in the wells**
- a. Determine the number of Microwell Strips required to test the desired number of samples plus **Microwell Strips** for running blanks and standards (**coloured**). 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 -20 °C sealed tightly. Place microwell strips containing the standard curve in position A1/A2 to H1/H2 (see Fig. 1).
- b. Add 150 µl of **distilled water** in duplicate to all standard wells. (A1, A2 to G1, G2)

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 (2500 pg/ml) | Standard 1 (2500 pg/ml) | Sample 1 | Sample 1 |
| B | Standard 2 (1250 pg/ml) | Standard 2 (1250 pg/ml) | Sample 2 | Sample 2 |
| C | Standard 3 (625 pg/ml) | Standard 3 (625 pg/ml) | Sample 3 | Sample 3 |
| D | Standard 4 (312.5 pg/ml) | Standard 4 (312.5 pg/ml) | Sample 4 | Sample 4 |
| E | Standard 5 (156.3 pg/ml) | Standard 5 (156.3 pg/ml) | Sample 5 | Sample 5 |
| F | Standard 6 (78.1 pg/ml) | Standard 6 (78.1 pg/ml) | Sample 6 | Sample 6 |
| G | Standard 7 (39 pg/ml) | Standard 7 (39 pg/ml) | Sample 7 | Sample 7 |
| H | Blank | Blank | Sample 8 | Sample 8 |

- c. Add 150 μ l of **distilled water**, in duplicate, to the blank wells.
- d. Add 100 μ l of **distilled water** to the sample wells.
- e. Add 50 μ l of each **Sample**, in duplicate, to the designated wells.
- f. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 3 hours on a microplate shaker at 200 rpm.
- g. Remove Plate Cover and empty wells. Wash the microwell strips three times with approximately 400 μ l **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, empty wells and 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. Pipette 100 μ l of **TMB Substrate Solution** to all wells, including the blank wells.
- i. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes, if available on a rotator set at 100 rpm. Avoid direct exposure to intense light.
The colour development on the plate should be monitored and the substrate reaction stopped (see point j. 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 620nm. The substrate reaction should be stopped as soon as an OD of 0.6 – 0.65 is reached.
- j. Stop the enzyme reaction by quickly pipetting 100 μ l 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.
- k. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the sICAM-1 standards.

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 mL-10 concentration on the abscissa. Draw a best fit curve through the points of the graph.
- To determine the concentration of circulating mL-10 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 mL-10 concentration.

***Samples have been diluted 1:2, thus the concentration read from the standard curve must be multiplied by the respective dilution factor.**

It is suggested that each testing facility establishes a control sample of known mL-10 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this 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. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

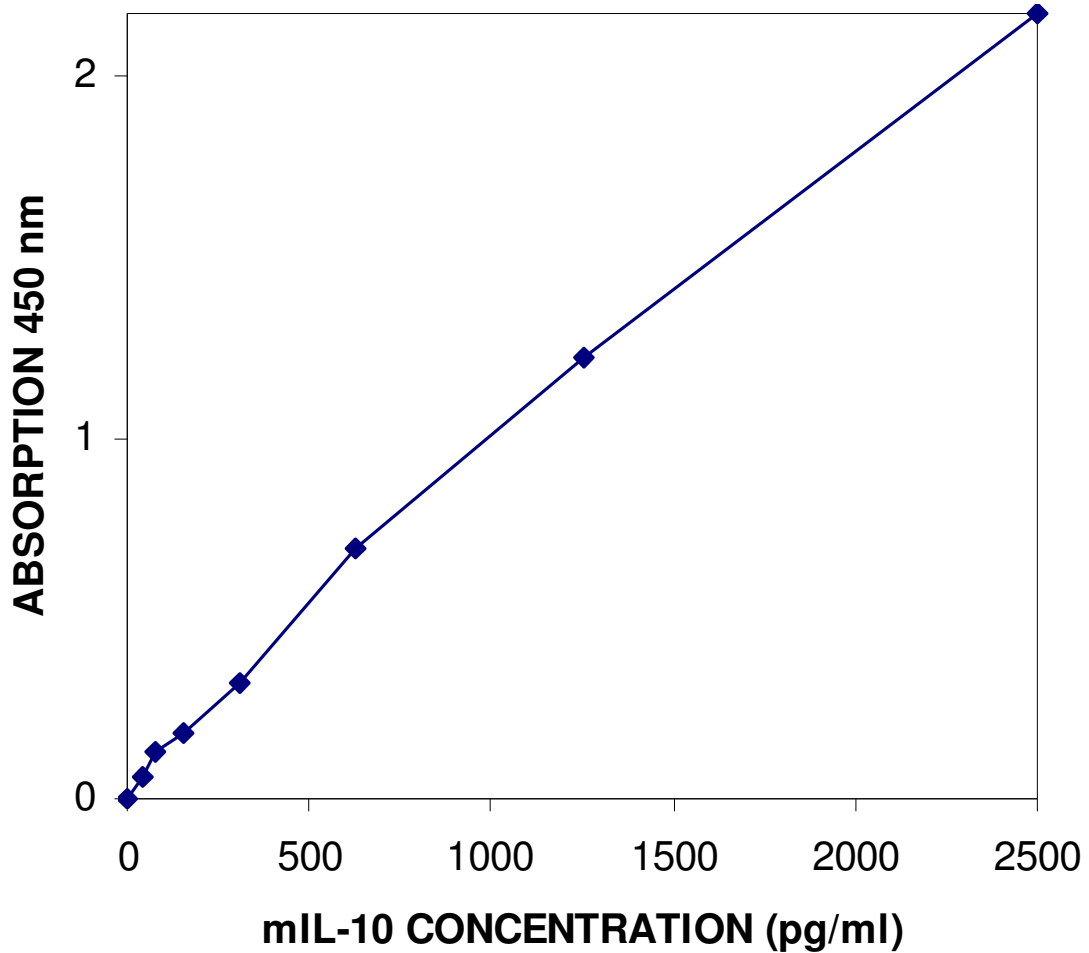
* N.B: There is a common dilution factor for standards and samples due to the conjugate which must then be included in the calculation. Both the standard and the samples contribute 100 µl to the final volume per well. For the sample wells these 100 µl are composed of 50 µl of sample diluent plus 50 µl of the sample. This is a 1:2 dilution. The remaining 50 µl to give 150 µl are due to the addition of 50 µl conjugate to all wells.

Filling scheme of wells:

Standard wells: 100 µl standard and 50 µl conjugate result in 150 µl reconstitution volume

Sample wells: 50 µl sample diluent and 50 µl conjugate result in 100 µl reconstitution volume, addition of 50 µl sample (50 µl+50 µl = 1:2 dilution)

Figure 2. Representative standard curve for mL-10 ELISA. mL-10 was diluted in serial two-fold steps in Sample Diluent, symbols represent the mean of three parallel titrations. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.



Typical data using the mL-10 Instant ELISA

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

| Standard | mL-10 Concentration (pg/ml) | O.D. (450 nm) | O.D. Mean | C.V. (%) |
|----------|-----------------------------------|------------------|--------------|-------------|
| 1 | 2500 | 2.387 | 2.254 | 8.4 |
| | 2500 | 2.12 | | |
| 2 | 1250 | 1.327 | 1.301 | 2.8 |
| | 1250 | 1.275 | | |
| 3 | 625 | 0.784 | 0.769 | 2.9 |
| | 625 | 0.753 | | |
| 4 | 312.5 | 0.391 | 0.400 | 3.2 |
| | 312.5 | 0.409 | | |
| 5 | 156.3 | 0.247 | 0.258 | 6.0 |
| | 156.3 | 0.269 | | |
| 6 | 78.1 | 0.196 | 0.207 | 7.2 |
| | 78.1 | 0.217 | | |
| 7 | 39.1 | 0.128 | 0.137 | 9.3 |
| | 39.1 | 0.146 | | |
| Blank | | 0.081 | 0.079 | |
| | | 0.077 | | |

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 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 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.

13. PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection of mL-10 defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 5.28 pg/ml (mean of 6 independent assays).

B. Reproducibility

a. Intra-assay

Reproducibility within the assay was evaluated in independent experiments. The overall intra-assay coefficient of variation has been calculated to be 6.7%.

b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in independent experiments by three technicians. The overall inter-assay coefficient of variation has been calculated to be 10.1%.

C. Spike Recovery

The spike recovery was evaluated by spiking four levels of mL-10 into pooled normal murine serum. Recoveries were determined in two independent experiments with 4 replicates each. Observed values showed an overall mean recovery of 93%.

D. Dilution Parallelism

Murine serum spiked with different levels of mL-10 was assayed at four serial twofold dilutions with 4 replicates each. Experiments showed an overall mean recovery of 95 %.

E. Sample Stability

a. Freeze-Thaw Stability

Aliquots of spiked serum were stored frozen at -20°C and thawed up to 5 times, and IL-10 levels determined. There was no significant loss of IL-10 by freezing and thawing up to 5 times.

b. Storage Stability

Aliquots of spiked serum were stored at -20°C , $2-8^{\circ}\text{C}$, room temperature (RT) and at 37°C , and the IL-10 level determined after 24 h. There was no significant loss of IL-10 immunoreactivity during storage at -20°C , 4°C and room temperature. Storage at 37°C gave rise to about 50 % loss of IL-10 immunoreactivity.

F. Specificity

The interference of circulating factors of the immune systems was evaluated by spiking these proteins at physiologically relevant concentrations into a IL-10 positive serum. There was no detectable cross reactivity.

G. Expected Serum Values

There are no detectable IL-10 levels found in healthy mice. Elevated IL-10 levels depend on the type of immunological disorder.

14. BIBLIOGRAPHY

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15. ORDERING INFORMATION

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Cat.No. BMS614INST mouse IL-10 Instant ELISA

16. REAGENT PREPARATION SUMMARY**Wash Buffer**

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

17. TEST PROTOCOL SUMMARY

- Place standard strip in position A1/A2 to H1/H2.
- Add 150 µl **distilled water**, in duplicate, to all standard wells
- Add 150 µl **distilled water**, in duplicate, to the blank wells
- Add 100 µl **distilled water**, in duplicate, to the sample wells
- Add 50 µl **Sample**, in duplicate, to designated wells
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C) on microplate shaker
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for 10 minutes at room temperature (18° to 25°C) on microplate shaker
- Add 100 µl **Stop Solution** to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

Note: *Samples have been diluted 1:2, thus the concentration read from the standard curve must be multiplied by the respective dilution factor (x2).

* N.B: There is a common dilution factor for standards and samples due to the conjugate which must then be included in the calculation. Both the standard and the samples contribute 100 µl to the final volume per well. For the sample wells these 100 µl are composed of 50 µl of sample diluent plus 50 µl of the sample. This is a 1:2 dilution. The remaining 50 µl to give 150 µl are due to the addition of 50 µl conjugate to all wells.

Filling scheme of wells:

Standard wells: 100 µl standard and 50 µl conjugate result in 150 µl reconstitution volume

Sample wells: 50 µl sample diluent and 50 µl conjugate result in 100 µl reconstitution volume, addition of 50 µl sample (50 µl+50 µl = 1:2 dilution)