PRODUCT INFORMATION AND MANUAL

human IL-10
Instant ELISA

BMS215INST

Enzyme-linked immunosorbent assay for quantitative

detection of human IL-10.

For research use only.

Not for diagnostic or therapeutic procedures.

128 Tests

human IL-10
BMS215INST

Bender MedSystems GmbH
Campus Vienna Biocenter 2
A-1030 Vienna, Austria, Europe

TABLE OF CONTENTS

1 Intended Use

The human IL-10 Instant ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human Interleukin-10 in cell culture supernatants, human serum, plasma, urine, synovial fluid, amniotic fluid or other body fluids. The human IL-10 Instant ELISA is **for research use only. Not for 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 this cytokine is a potent suppressor of the effector 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 primary structure of human IL-10 has been determined by cloning the cDNA encoding the cytokine (15). The corresponding protein exerts 160 amino acids with a predicted molecular mass of 18.5 kDa (8, 15). Based on its primary structure, IL-10 is a member of the four *-helix bundle family of cytokines (11). In solution human IL-10 is a homodimer with an apparent molecular mass of 39 kDa (14). Although it contains an N-linked glycosylation site, it lacks detectable carbohydrates (15). Recombinant protein expressed in E. coli thus retains all known biological activities. The human IL-10 gene is located on chromosome 1 and is present as a single copy in the genome (6).
The human IL-10 exhibits strong DNA and amino acid sequence homology to the murine 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.

The immunosuppressive properties of IL-10 (4) suggest a possible clinical use of IL-10 in suppressing rejections of grafts after organ transplantations. IL-10 can furthermore exert strong anti-inflammatory activities (4).

IL-10 in disease:

IL-10 expression was shown to be elevated in parasite infections like in Schistosoma mansoni (7), Leishmania (5), Toxoplasma gondii (12) and Trypanosoma (13) infection.

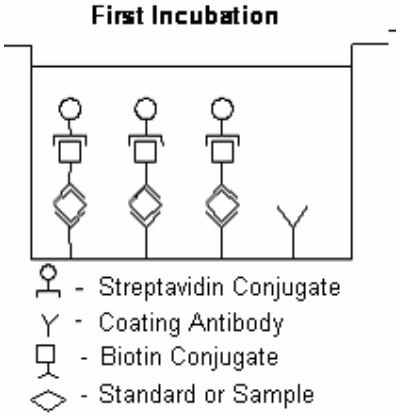
Furthermore, high IL-10 expression was detected in mycobacterial infections as shown for Mycobacterium leprae (3), Mycobacterium tuberculosis (2) and Mycobacterium avium infections.

High expression levels of IL-10 are also found in retroviral infections inducing immunodeficiency (10).

3 Principles of the Test

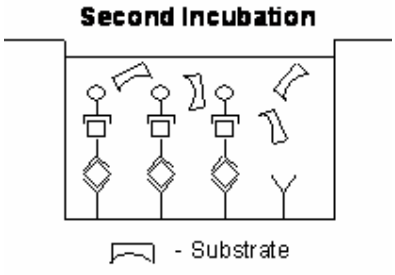
An anti-human IL-10 monoclonal coating antibody is adsorbed onto microwells. Human IL-10 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated monoclonal anti-human IL-10 antibody binds to human IL-10 captured by the first antibody. Streptavidin-HRP binds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

Figure 1



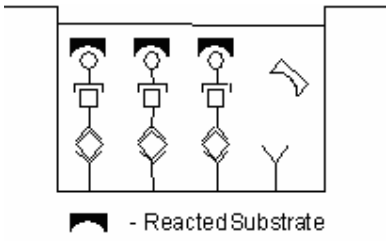
Following incubation unbound biotin conjugated anti human IL-10 and Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Figure 2



A coloured product is formed in proportion to the amount of soluble human IL-10 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven human IL-10 standard dilutions and human IL-10 sample concentration determined.

Figure 3



4 Reagents Provided

1 aluminium pouch with **a Microwell Plate coated with Monoclonal Antibody** (murine) to human IL-10, **Biotin-Conjugate** (anti-IL-10 monoclonal antibody), Sample diluent and **Streptavidin-HRP**, lyophilized

2 aluminium pouches with a **human IL-10 Standard curve (coloured)**

1 bottle (25 ml) **Wash Buffer Concentrate** 20x (phosphate-buffered saline with 1% Tween 20)

1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)

1 vial (12 ml) **Sample Diluent** (Use when an external predilution of the samples is needed)

1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)

2 adhesive **Plate Covers**

5 Storage Instructions

Store ELISA plate and Standard curves or whole kit at -20°C. The plate and the standard curves can also be removed, stored at -20°C, remaining kit reagents can be 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, human serum, plasma, urine, synovial fluid, amniotic fluid or other body fluids are suitable for use in the assay. Remove the serum or plasma from the clot or red cells as soon as possible after clotting and separation.

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

Samples must be stored frozen at -20°C to avoid loss of bioactive human IL-10. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13).
Avoid repeated freeze-thaw cycles. Prior to assay, frozen serum or plasma should be brought to room temperature slowly and mixed gently.

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 substrate reagent.

- 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 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 and Samples

9.1 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 to 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 Wash Buffer is stable for 30 days.

10 Test Protocol

- **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 400 µl 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 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 Table 1).

b. Add 100 µl of distilled water to the sample wells.

c. Add distilled water to all standard and blank wells as indicated on the label of the standard strips (A1, A2 to H1, H2).

Table 1

Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (200.0 pg/ml)	Standard 1 (200.0 pg/ml)	Sample 1	Sample 1
B	Standard 2 (100.0 pg/ml)	Standard 2 (100.0 pg/ml)	Sample 2	Sample 2
C	Standard 3 (50.0 pg/ml)	Standard 3 (50.0 pg/ml)	Sample 3	Sample 3
D	Standard 4 (25.0 pg/ml)	Standard 4 (25.0 pg/ml)	Sample 4	Sample 4
E	Standard 5 (12.5 pg/ml)	Standard 5 (12.5 pg/ml)	Sample 5	Sample 5
F	Standard 6 (6.3 pg/ml)	Standard 6 (6.3 pg/ml)	Sample 6	Sample 6
G	Standard 7 (3.1 pg/ml)	Standard 7 (3.1 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

d. Add 50 µl of each **Sample**, in duplicate, to the designated wells and mix the contents.

e. Cover with a **Plate Cover** and incubate at room temperature (18ºC to 25ºC) for 3 hours, if available on a microplate shaker at 200 rpm.

f. Remove **Plate Cover** and empty wells. Wash the microwell strips 6 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 no longer than 15 minutes. Do not allow wells to dry.

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

h. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

   The colour development on the plate should be monitored and the substrate reaction stopped (see point i. of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour 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 colour.
   Alternatively the colour 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 0.6 – 0.65.

i. 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.

j. 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 human IL-10 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 human IL-10 concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating human IL-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 human IL-10 concentration.

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

- It is suggested that each testing facility establishes a control sample of known human IL-10 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 4. 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 samples due to the conjugate which must then be included in the calculation. The samples contribute 100 µl to the final volume per well. 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.
50 µl sample diluent and 50 µl conjugate results in 100 µl reconstitution volume, addition of 50 µl sample (50 µl + 50 µl = 1:2 dilution)

Figure 4

Representative standard curve for human IL-10 Instant ELISA. Human IL-10 was diluted in serial 2-fold steps in Sample Diluent, each symbol represents the mean of 3 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.

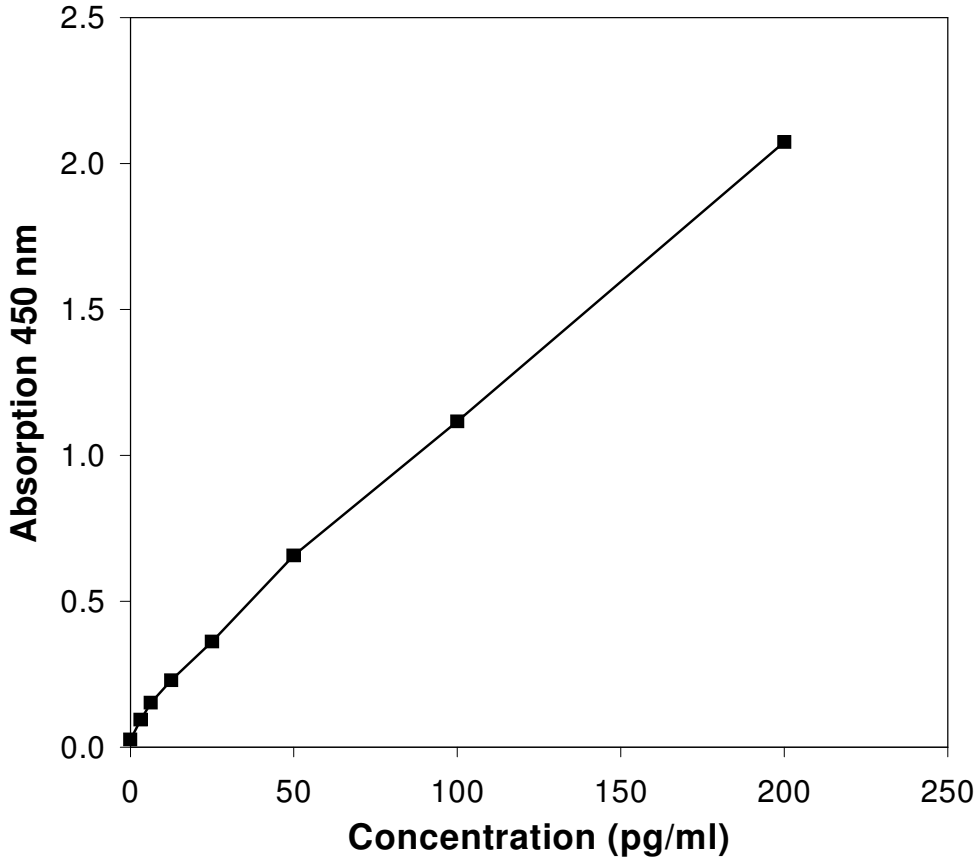


Table 2
Typical data using the human IL-10 INSTANT ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	human IL-10 Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	200.0	2.119	2.074	3.1
	200.0	2.029		
2	100.0	1.158	1.116	5.3
	100.0	1.074		
3	50.0	0.652	0.657	1.1
	50.0	0.662		
4	25.0	0.381	0.362	7.4
	25.0	0.343		
5	12.5	0.226	0.229	1.9
	12.5	0.232		
6	6.3	0.154	0.152	2.3
	6.3	0.149		
7	3.1	0.090	0.094	5.3
	3.1	0.097		
Blank	0	0.025	0.026	
	0	0.027		

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 detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the Sample.

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of human IL-10 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.66 pg/ml (mean of 6 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of human IL-10. 2 standard curves were run on each plate. Data below show the mean human IL-10 concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 6.1%.

Table 3
The Mean human IL-10 concentration and the coefficient of variation for each sample.

Positive Sample	Experiment	human IL-10 Concentration (pg/ml)	Coefficient of Variation (%)
1	1	208	5
	2	206	5
	3	214	7
2	1	112	8
	2	114	10
	3	110	7
3	1	61	6
	2	55	10
	3	50	4
4	1	175	3
	2	204	7
	3	233	2
5	1	81	2
	2	107	6
	3	98	10
6	1	48	10
	2	54	7
	3	55	7
7	1	21	5
	2	20	4
	3	26	4

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments by 3 technicians. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of human IL-10. 2 standard curves were run on each plate. Data below (see Table 4) show the mean human IL-10 concentration and the coefficient of variation calculated on 18 determinations of each sample. The calculated overall coefficient of variation was 9.1%.

Table 4
The mean human IL-10 concentration and the coefficient of variation calculated on 18 determinations of each sample.

Sample	human IL-10 Concentration (pg/ml)	Coefficient of Variation (%)
1	209	2.0
2	112	1.8
3	55	10.1
4	204	14.3
5	95	13.8
6	52	7.7
7	23	14.1

13.3 Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human IL-10 into pooled normal human serum. Recoveries were determined in 3 independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments. Average recovery ranged from 80% to 107% with an overall mean recovery of 86%.

13.4 Dilution Parallelism

4 serum samples with different levels of human IL-10 were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged between 81% to 121% with an overall mean recovery of 102% (see Table 5).

Table 5

		Human IL-10 Concentration (pg/ml)		
Sample	Dilution	Expected Value	Observed Value	% Recovery of Exp. Val.
1	1:2	--	299.6	--
	1:4	149.8	181.8	121.4
	1:8	90.9	105.1	115.6
	1:16	52.5	45.7	86.9
2	1:2	--	229.1	--
	1:4	114.5	130.1	113.6
	1:8	65.0	65.4	100.6
	1:16	32.7	30.0	91.6
3	1:2	--	164.9	--
	1:4	82.4	93.4	113.3
	1:8	46.7	49.0	104.9
	1:16	24.5	19.8	80.9
4	1:2	--	90.7	--
	1:4	45.4	49.1	108.2
	1:8	24.6	21.1	85.7
	1:16	10.5	10.7	101.3

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed 5 times, and the human IL-10 levels determined. There was no significant loss of human IL-10 immunoreactivity by freezing and thawing.

13.5.2 Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the human IL-10 level determined after 24 h. There was no significant loss of human IL-10 immunoreactivity during storage under above conditions.

13.6 Comparison of Serum and Plasma

From two individuals, serum as well as EDTA and citrate, and heparin plasma obtained at the same time point were evaluated. Human IL-10 concentrations were not significantly different and therefore all these body fluids are suitable for the assay. It is nevertheless highly recommended to assure the uniformity of blood preparations.

13.7 Specificity

To define the specificity of this ELISA several proteins were tested for cross reactivity. There was no cross reactivity observed.

13.8 Expected Values

There are no detectable human IL-10 levels found in healthy blood donors. Elevated human IL-10 levels depend on the type of immunological disorder and the severity of the disease.

13.9 Calibration

This immunoassay is calibrated with highly purified recombinant human IL-10, which has been evaluated against the International Reference Standard NIBSC 93/722 and has been shown to be equivalent. NIBSC 93/722 is quantitated in International Units (IU), 1IU corresponding to 200 pg human IL-10.

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For literature update refer to **www.bendermedsystems.com/44.html**

15 Ordering Information

For orders please contact:

Europe-Headquarters
Bender MedSystems GmbH
Campus Vienna Biocenter 2
A-1030 Vienna, Austria, Europe
phone: +43 1 796 40 40 ext. 114
fax: +43 1 796 40 40 ext. 400
e-mail: order@bendermedsystems.com

USA
Bender MedSystems, Inc.
849 Hinckley Road
Burlingame, CA 94010, USA
toll-free phone: +1 (866) 952 2112
toll-free fax: +1 (877) 952 2112
phone: +1 (650) 952 2112
fax: +1 (650) 952 2252

For technical information please contact:

e-mail: techserv@bendermedsystems.com
www.bendermedsystems.com

Cat.No. BMS215INST human IL-10 INSTANT ELISA

16 Reagent Preparation Summary

16.1 Wash Buffer

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

17 Test Protocol Summary

- Place standard strips in position A1/A2 to H1/H2.
- Add 100 µl **distilled water** to sample wells.
- Add **distilled water**, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 50 µl **Sample** to designated wells.
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C) on a microplate shaker at 200 rpm.
- Empty and wash microwell strips 6 times with 400 µl **Wash Buffer**.
- Add 100 µl of **TMB Substrate Solution** to all wells including blank wells.
- Incubate the microwell strips for about 10 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: Samples have been diluted 1:2, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 2).