コスモ・バイオ株式会社

PRODUCT INFORMATION AND MANUAL

human IL-2 ELISA Version 2

BMS221

Enzyme-linked immunosorbent assay for quantitative detection of human Interleukin-2.

For research use only.

Not for diagnostic or therapeutic procedures.

96 Tests

human IL-2
BMS221

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member of the mercure group

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1. INTENDED USE

The IL-2 ELISA is an enzyme-linked immunosorbent assay for quantitative detection of human Interleukin-2 in cell culture supernatants, human serum, plasma or other body fluids. **The IL-2 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2. SUMMARY

Interleukin-2 (IL-2) plays a central role in the activation and proliferation of lymphocytes that have been primed by antigens (14). IL-2 plays a pivotal role in for the expansion of most T-cells, natural killer cells and B-cells during certain phases of their response.
IL-2 is a 15 kDa glycoprotein encoded by a single gene located in the q26-28 region of human chromosome 4 (11, 15). The cDNA deduced polypeptide consists of 153 amino acids (5, 16).
IL-2 gene expression is regulated at the transcriptional level by several activation pathways. Antigen-specific proliferation of helper and cytotoxic T-lymphocytes following stimulation is critically dependent on IL-2 expression, secretion, and binding to receptors for IL-2 induced in an autocrine fashion on the surface of T-cells (12).

Apart from its most important role to mediate antigen-specific T-lymphocyte proliferation (18), IL-2 modulates the expression of interferon-γ (10) and major histocompatibility antigens (3), stimulates proliferation and differentiation of activated B-cells (17), augments natural killer cell activity (4) and inhibits granulocyte-macrophage colony formation (8).

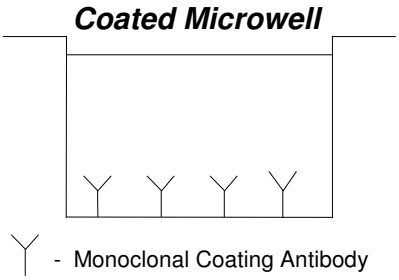
Alterations in the ability of T-cells to synthesize IL-2 have been observed in physiologic and pathologic states.

Because of the central role of IL-2 in immune response, IL-2 turned out to be a very important molecule for diagnostic and therapeutic implications.
IL-2 displays antitumoral effects, thus being used in cancer therapy (18).

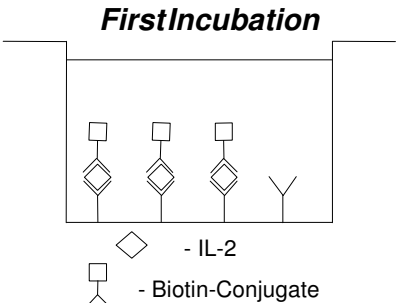
Monitoring of IL-2 levels in serum provides more detailed insights in several pathological situations such as **cancer** (13), **infectious diseases** (13), **transplant rejection** (1, 6), **multiple sclerosis** (2), **rheumatoid arthritis** (9), **systemic lupus erythematosus** (7) and **type I diabetes.**

3. PRINCIPLES OF THE TEST

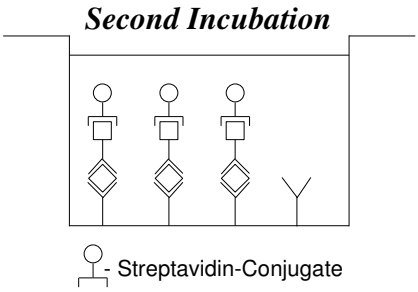
An anti-IL-2 monoclonal coating antibody is adsorbed onto microwells.

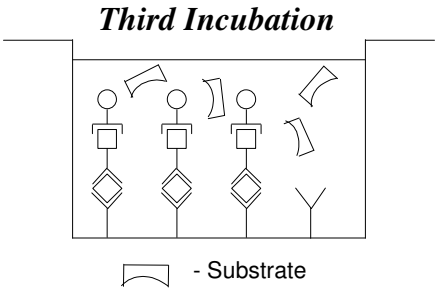


IL-2 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated polyclonal anti-IL-2 antibody is added and binds to IL-2 captured by the first antibody.

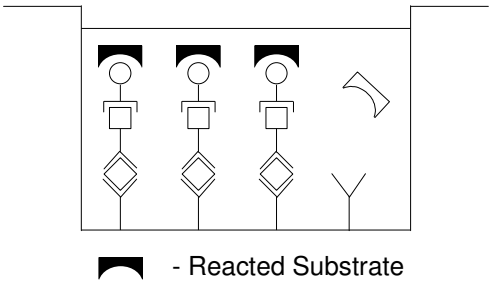


Following incubation unbound biotin conjugated anti-IL-2 is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-IL-2. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.





A coloured product is formed in proportion to the amount of IL-2 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 IL-2 standard dilutions and IL-2 sample concentration deter-mined.



4. REAGENTS PROVIDED

1 aluminium pouch with **a Microwell Plate coated with Monoclonal Antibody** (murine) to human IL-2

1 vial (100 µl) **Biotin-Conjugate** anti-IL-2 polyclonal (rabbit) antibody

1 vial (150 µl) **Streptavidin-HRP**

2 vials **IL-2 Standard, lyophilized, 2400 pg/ml upon reconstitution** (adjusted to 1st International Standard for Interleukin-2 (Human) (established 1987) no. 86/504, 100 IU ≡ 7.6 ng IL-2).

1 vial **Control low**

1 vial **Control high**

1 bottle (50 ml) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20)

1 bottle (50 ml) **Sample Diluent**

1 vial (7 ml) **Substrate Solution I** (tetramethyl-benzidine)

1 vial (7 ml) **Substrate Solution II** (0.02 % buffered hydrogen peroxide)

1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)

1 vial (0.4 ml each) ***Blue-Dye, Green-Dye, Red-Dye***

4 adhesive **Plate Covers**

Reagent Labels

5. STORAGE INSTRUCTIONS

Store kit reagents between 2° and 8℃. Immediately after use remaining reagents should be returned to cold storage (2° to 8℃). 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, EDTA, heparin and citrate 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℃ and separated rapidly before storing at -20℃ to avoid loss of bioactive IL-2. If samples are to be run within 24 hours, they may be stored at 2° to 8℃. Avoid repeated freeze-thaw cycles.

For stability and suitability of samples refer to 13. E, and F.

7. MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes
- 10 µl to 1,000 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.

- Reagents containing thimerosal as preservative may be toxic if ingested.

- Avoid contact of substrate solutions with oxidizing agents and metal.

- Avoid splashing or generation of aerosols.

- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.

- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.

- Exposure to acids will inactivate the conjugate.

- Glass-distilled water or deionized water must be used for reagent preparation.

- Substrate solutions 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℃.

- 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

A. Wash Buffer

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

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 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℃. Please note that the Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

B. Preparation of Biotin-Conjugate

Make a 1:100 dilution with **Sample Diluent** in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Sample Diluent (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

C. Preparation of IL-2 Standard

Reconstitute IL-2 Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Make sure the contents entirely dissolve by gentle swirling. The standard solution obtained is 2400 pg/ml.

D. Preparation of Streptavidin-HRP

Make a 1:100 dilution of the concentrated **Streptavidin-HRP** solution in **Sample Diluent** as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Sample Diluent (ml)
1 - 6	0.06	6
1 - 12	0.12	12

E. Preparation of controls

Solubilize by adding 800µl Sample Diluent to each vial labeled human IL-2 control. Swirl or mix gently to ensure complete and homogeneous solubilization. Add directly to the plate, no further dilution necessary.
For control range please refer to certificate of analysis or vial label. Store reconstituted controls aliquoted at –20℃. Avoid repeated freeze and thaw cycles.

F. TMB Substrate Solution

Using clean pipettes and containers known to be metal free, dispense an equal volume of **Substrate Solution I** into **Substrate Solution II** and swirl gently to mix. The TMB Substrate Solution may develop a yellow tinge over time. This does not seem to affect product performance. A blue colour present in the TMB Substrate Solution, however, indicates that it has been contaminated and must be discarded. The TMB Substrate Solution must be used within a few minutes after mixing. Warm to room temperature before use. Avoid direct exposure of TMB reagents to intense light and oxidizing agents during storage or incubation.

Substrate preparation according to assay size:

Number of Strips	Substrate Solution I (ml)	Substrate Solution II (ml)
1 - 6	3.0	3.0
1 - 12	6.0	6.0

G. Addition of Colour-giving Dyes

In order to help our customers to avoid any mistakes in pipetting the Bender MedSystems ELISAs, Bender MedSystems now offers a new tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours to each step of the ELISA procedure.
This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.
Alternatively, the dye solutions from the stocks provided (***Blue-Dye, Green-Dye, Red-Dye***) can be added to the reagents according to the following guidelines:

1. Diluent:

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

5 ml Diluent	20 µl ***Blue-Dye***
12 ml Diluent	48 µl ***Blue-Dye***
50 ml Diluent	200 µl ***Blue-Dye***

2. Biotin-Conjugate:

Before dilution of the concentrated conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Diluent used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet, preparation of Biotin-conjugate.
gate is supplied ready to use, directly add the ***Green-Dye*** at a dilution of 1:100.

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

3. Streptavidin-HRP:

Before dilution of the concentrated Streptavidin-HRP, add the ***Red-Dye*** at a dilution of 1:250 (see table below) to the Diluent used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the instruction booklet, preparation of Streptavidin-HRP.

6 ml Diluent	24 µl ***Red-Dye***
12 ml Diluent	48 µl ***Red-Dye***

10. TEST PROTOCOL

a. Prepare reagents immediately before use and mix them thoroughly without foaming.

b. **Dilution of samples:**

Serum or **plasma** samples are applied undiluted. 2 x 100 µl serum or plasma are needed for duplicate measurement.

Because IL-2 levels in **Cell Culture Supernatants** may vary considerably, it is not possible to recommend a predetermined dilution factor. Optimal dilution has to be determined for each individual sample. For unknown cell culture samples it is useful to analyze undiluted as well as prediluted samples (e.g. 1:20 - 1:50) in parallel, thereby covering a wider range in one assay.
Cell culture supernatants with very high concentrations of IL-2 require high dilutions (e.g. up to 1:2000) in order to be measured correctly. Such samples should be **prediluted** in the respective **Culture Medium**. **Final dilution** should be performed in Sample Diluent similar to one of the examples shown below.

Some examples of generating dilutions of cell culture supernatants			
Dilution	**Sample Volume**	**Sample Diluent**	**to calculate the concentration of original sample, result must be multiplied by:**
1 : 5	50 µl Sample	200 µl Sample Diluent	x 5
1 : 10	25 µl Sample	225 µl Sample Diluent	x 10
1 : 50	10 µl Sample	490 µl Sample Diluent	x 50
1 : 100	A: 10 µl Sample B: 25 µl predilution "A"	90 µl Sample Diluent 225 µl Sample Diluent	x 100
1 : 1000	A: 10 µl Sample B: 25 µl predilution "A"	990 µl **Culture Medium** 225 µl Sample Diluent	x 1000
1 : 2000	A: 10 µl Sample B: 10 µl predilution "A"	390 µl **Culture Medium** 490 µl Sample Diluent	x 2000

c. 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 IL-2 from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

d. Wash the microwell strips twice with approximately 300 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, 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.

e. Add 100 µl of **Sample Diluent** in duplicate to all standard wells. Prepare standard dilutions by pipetting 100 µl of reconstituted (Refer to preparation of reagents, 9.C.) **IL-2 Standard**, in duplicate, into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100 µl to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of IL-2 standard dilutions ranging from 1200 to 19 pg/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of IL-2 standard dilutions:

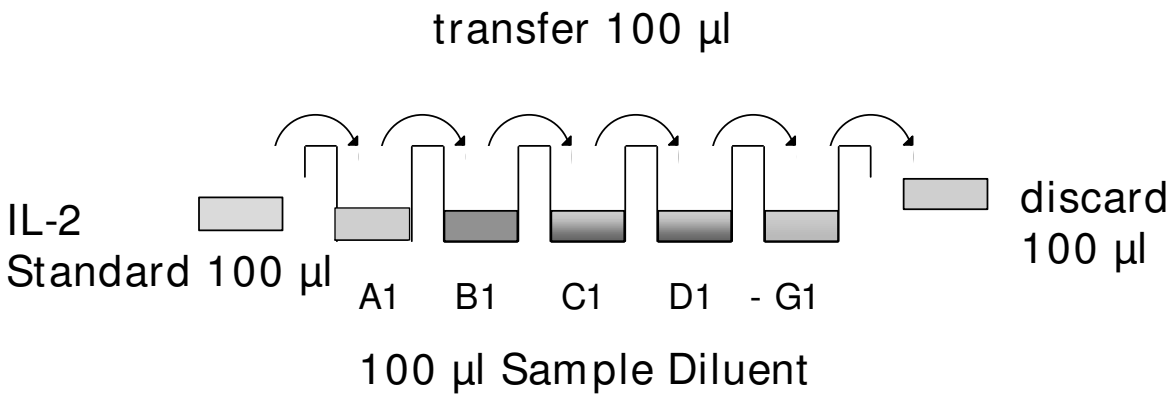


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (1200 pg/ml)	Standard 1 (1200 pg/ml)	Sample 1	Sample 1
B	Standard 2 (600 pg/ml)	Standard 2 (600 pg/ml)	Sample 2	Sample 2
C	Standard 3 (300 pg/ml)	Standard 3 (300 pg/ml)	Sample 3	Sample 3
D	Standard 4 (150 pg/ml)	Standard 4 (150 pg/ml)	Sample 4	Sample 4
E	Standard 5 (75 pg/ml)	Standard 5 (75 pg/ml)	Sample 5	Sample 5
F	Standard 6 (38 pg/ml)	Standard 6 (38 pg/ml)	Sample 6	Sample 6
G	Standard 7 (19 pg/ml)	Standard 7 (19 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

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

g. Add 100 µl of each **Sample**, in duplicate, to the designated wells.

h. Prepare **Biotin-Conjugate**. (Refer to preparation of reagents)

i. Add 50 µl of diluted **Biotin-Conjugate** to all wells, including the blank wells.

j. Cover with a **Plate Cover** and incubate at room temperature (18° to 25℃) for 2 hours on a microplate shaker set at 200 rpm.

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

l. Prepare **Streptavidin-HRP**. (Refer to preparation of reagents)

m. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.

n. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 1 hour on a microplate shaker at 200 rpm.

o. Prepare **TMB Substrate Solution** a few minutes prior to use (Refer to preparation of reagents).

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

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

r. 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 s. 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.

s. 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.

t. 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 IL-2 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 IL-2 concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating IL-2 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 IL-2 concentration.

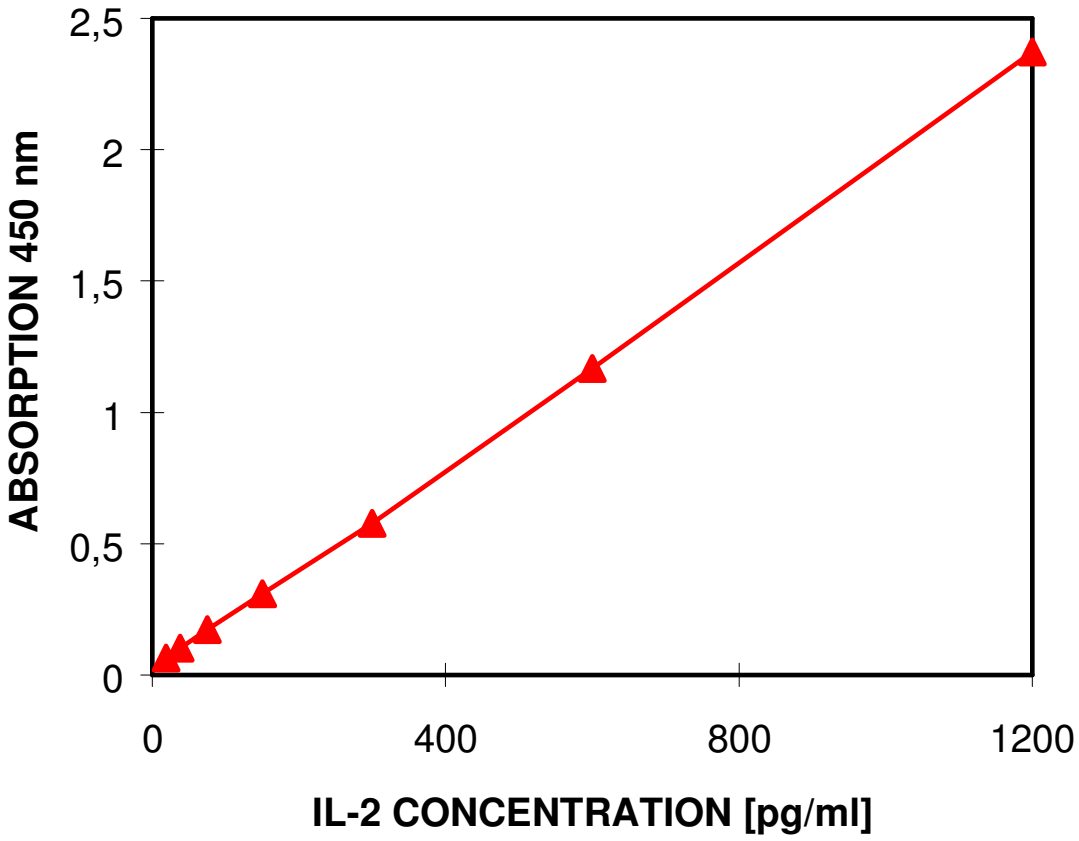
  If samples have been diluted according to the instructions given in this manual (e.g. cell culture supernatants), the concentration read from the standard curve must be multiplied by the respective dilution factor.

  Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low IL-2 levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual IL-2 level.

  It is suggested that each testing facility establishes a control sample of known IL-2 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 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for IL-2 ELISA. IL-2 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 IL-2 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	IL-2 Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	1200	2.294	2.372	3.3
	1200	2.450		
2	600	1.166	1.167	0.2
	600	1.168		
3	300	0.592	0.577	2.6
	300	0.562		
4	150	0.313	0.310	1.0
	150	0.307		
5	75	0.171	0.172	0.4
	75	0.173		
6	38	0.105	0.102	2.0
	38	0.100		
7	19	0.066	0.063	4.8
	19	0.060		
Blank	0	0.029	0.028	
	0	0.026		

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 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.

13. PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection of IL-2 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 9.9 pg/ml (mean of 6 independent assays).

B. Reproducibility

a. Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 4 serum and 4 cell culture samples containing different concentrations of IL-2. Two standard curves were run on each plate. Data below show the mean IL-2 concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 5.2 %.

Positive Sample	Experiment	IL-2 Concentration (pg/ml)	Coefficient of Variation (%)
1	1	251	7.9
	2	283	2.2
	3	238	8.8
2	1	150	5.6
	2	170	4.6
	3	128	6.9
3	1	336	7.4
	2	396	2.8
	3	332	5.6
4	1	127	4.5
	2	146	6.5
	3	123	4.6
5	1	202	3.4
	2	236	4.3
	3	202	4.8
6	1	78	5.9
	2	87	4.5
	3	68	6.5
7	1	241	2.8
	2	273	3.1
	3	264	4.4
8	1	168	6.2
	2	193	7.5
	3	169	4.8

b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by three technicians. Each assay was carried out with 6 replicates of 8 cell culture samples containing different concentrations of IL-2. Two standard curves were run on each plate. Data below show the mean IL-2 concentration and the coefficient of variation calculated on 18 determinations of each sample. The overall inter-assay coefficient of variation has been calculated to be 8 %.

Sample	IL-2 Concentration (pg/ml)	Coefficient of Variation (%)
1	257	7.5
2	149	11.4
3	355	8.3
4	132	7.6
5	213	7.5
6	78	9.9
7	259	5.3
8	177	6.6

C. Spike Recovery

The spike recovery was evaluated by spiking three levels of IL-2 into pooled normal human serum and citrate plasma, respectively. Recoveries were determined in two independent experiments with 4 replicates each. Observed values ranged from 71 to 93 % with an overall mean recovery of 78 %.

Experiment 1

IL-2 spiked in pooled serum at:	1000 pg/ml	300 pg/ml	100 pg/ml
	Recovery (%)		
	93	83	72

Experiment 2

IL-2 spiked in citrate plasma at:	1000 pg/ml	300 pg/ml	100 pg/ml
	Recovery (%)		
	81	73	71

D. Dilution Parallelism

Serum (1), citrate plasma (2) and two cell culture supernatants (3,4) spiked with different levels of IL-2 were assayed at four serial two-fold dilutions with 4 replicates each. In the table below the per cent recovery of expected values is listed. Recoveries ranged from 100 % to 115 % with an overall mean recovery of 107 %.

		IL-2 Concentration (pg/ml)		
Sample	Dilution	Expected Value	Observed Value	% Recovery of Exp. Value
1	--	--	654	--
	1:2	327	358	110
	1:4	179	180	101
	1:8	90	100	112
2	--	--	520	--
	1:2	260	260	100
	1:4	130	135	104
	1:8	67	71	106
3	1:10	--	9360	--
	1:20	4680	4993	107
	1:40	2340	2527	108
	1:80	1170	1326	113
4	1:10	--	6068	--
	1:20	3034	3238	107
	1:40	1517	1745	115
	1:80	759	805	106

E. Sample Stability

a. Freeze-Thaw Stability

Aliquots of serum and cell culture samples (unspiked or spiked) were stored frozen at -20℃ and thawed up to 5 times, and IL-2 levels determined. There was no significant loss of IL-2 by freezing and thawing up to 3 cycles of freezing and thawing. Further freeze-thaw cycles gave rise to about 20 % loss of IL-2 immunoreactivity.

b. Storage Stability

Aliquots of a serum and cell culture samples (spiked or unspiked) were stored at -20℃, 2-8℃, room temperature (RT) and at 37℃, and the IL-2 level determined after 24 h. There was no significant loss of IL-2 immunoreactivity during storage at -20℃, 4℃ and room temperature. Storage at 37℃ gave rise to about 20 % loss of IL-2 immunoreactivity..

F. Comparison of Serum and Plasma

From several individuals, serum as well as EDTA and citrate, and heparin plasma obtained at the same time point were evaluated. IL-2 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.

G. Specificity

The interference of circulating factors of the immune systems was evaluated by spiking these proteins at physiologically relevant concentrations into a IL-2 positive serum. There was no detectable cross reactivity.

H. Expected Serum Values

There are no detectable IL-2 levels found in healthy blood donors. Elevated IL-2 levels depend on the type of immunological disorder and the severity of disease.

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15. ORDERING INFORMATION

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Cat.No. BMS221 IL-2 ELISA

16. REAGENT PREPARATION SUMMARY

A. Wash Buffer

Add **Wash Buffer Concentrate** 20 x (50 ml) to 950 ml distilled water

B. Biotin-Conjugate

Make a 1:100 dilution according to the table.

Number of Strips	Biotin-Conjugate (ml)	Sample Diluent (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

C. Standard

Add the volume of **distilled water** as stated on the label of the vial of lyophilized **IL-2 Standard** as needed.

D. Streptavidin-HRP

Number of Strips	Streptavidin-HRP (ml)	Sample Diluent (ml)
1 - 6	0.06	6.0
1 - 12	0.12	12.0

E. TMB Substrate Solution

Number of Strips	Substrate Soloution I (ml)	Substrate Solution II (ml)
1 - 6	3.0	3.0
1 - 12	6.0	6.0

17. TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Sample Diluent**, in duplicate, to all standard wells
- Pipette 100 µl diluted **IL-2 Standard** into the first wells and create standard dilutions ranging from 1200 to 19 pg/ml by transferring 100 µl from well to well. Discard 100 µl from the last wells. Add 100 µl **Sample Diluent**, in duplicate, to the blank wells
- Add 100 µl **Sample**, in duplicate, to designated wells
- Prepare **Biotin-Conjugate**
- Add 50 µl of diluted **Biotin-Conjugate** to all wells
- Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C) on microplate shaker
- Prepare **Streptavidin-HRP**
- Empty and wash microwell strips 4 times with **Wash Buffer**
- Add 100 µl of diluted **Streptavidin-HRP** to all wells
- Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C) on microplate shaker
- Prepare **TMB Substrate Solution** few minutes prior to use
- Empty and wash microwell strips 4 times with **Wash Buffer**
- Add 100 µl of mixed **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for 10-20 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: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low IL-2 levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual IL-2 level.