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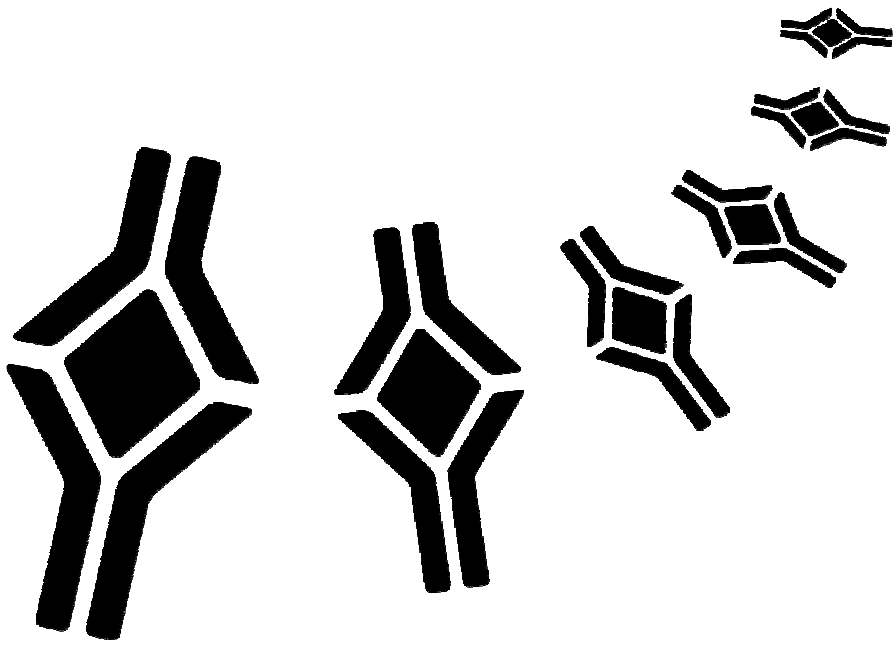


Monkey INTERLEUKIN-1β ELISA

Product Data Sheet

Cat. No.: RBMS645R

For Research Use Only

CONTENTS

This kit is manufactured by:
BioVendor – Laboratorní medicína, a.s.

Use only the current version of Product Data Sheet enclosed with the kit!

1 INTENDED USE

The monkey IL-1β ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of monkey IL-1β. **The monkey IL-1β ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

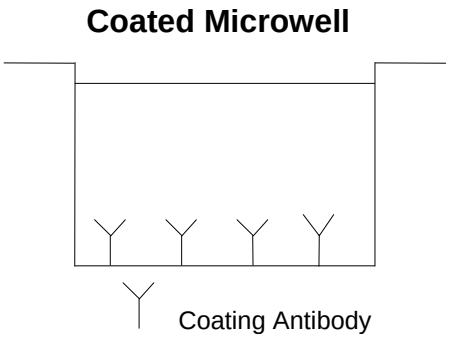
2 SUMMARY

Interleukin-1 (IL-1), originally described in 1972 as lymphocyte activating factor (LAF) for its effects on thymocytes (1), is a polypeptide cytokine with two molecular forms, IL-1α and IL-1β. Both forms appear to mediate identical ranges of biological activity which include synthesis of the acute phase proteins by hepatocytes, chemotaxis of polymorphonucleocytes, and release of polymorphonucleocytes from blood and bone marrow (2). These effects coined the acronym leukocyte endogenous mediator (LEM). Early researchers also called IL-1β endogenous pyrogen, and it has been shown to induce fever (3) and is thought to contribute to wasting of muscles (PIF, proteolysis inducing factor) (4). Other activities associated with IL-1 are the induction of Prostaglandin E2 by synovial cells and release of collagenase with resulting destruction of cartilage and bone resorption (catabolin, osteoclast activation factor) (5). In addition, IL-1, has multiple immunological functions including enhancement of IL-2 production by T cells and activation of B-cells (BAF) and thymocytes (6-8). A true pleiotrope, IL-1 may have tumoricidal activity via its release of IL-2 and interferon gamma and be indirectly antiviral by stimulating fibroblasts to release interferon beta (9, 10). In its role as mediator of sepsis, IL-1 has most recently been described as enhancing the growth of virulent E.coli (11).

3 PRINCIPLES OF THE TEST

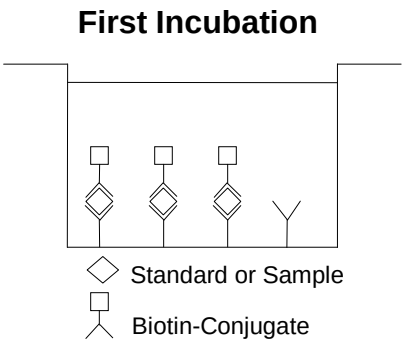
An anti-monkey IL-1β coating antibody is adsorbed onto microwells.

Figure 1



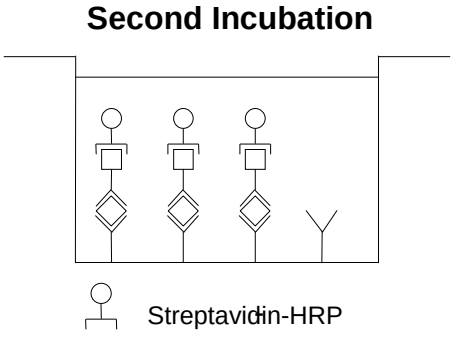
Monkey IL-1β present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-monkey IL-1β antibody is added and binds to monkey IL-1β captured by the first antibody.

Figure 2



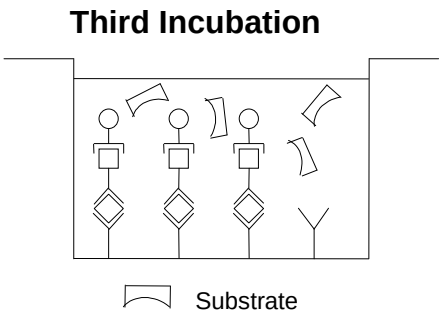
Following incubation unbound biotin-conjugated anti-monkey IL-1β antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-monkey IL-1β antibody.

Figure 3



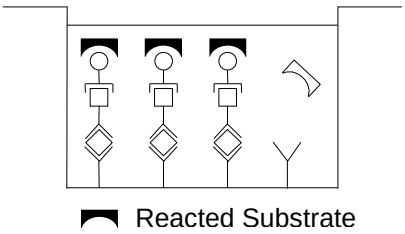
Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Figure 4



A coloured product is formed in proportion to the amount of monkey IL-1β 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 monkey IL-1β standard dilutions and monkey IL-1β sample concentration determined.

Figure 5



4 REAGENTS PROVIDED

1 aluminium pouch with a **Antibody Coated Microtiter Strips** with antibody to monkey IL-1β
1 vial (8 ml) **Biotin-Conjugate** anti-monkey IL-1β antibody
1 vial (75 µl) **Streptavidin-HRP**
2 vials monkey IL-1β **Standard** lyophilized, 400 U/ml upon reconstitution
1 vial (12 ml) **Standard Diluent**
1 vial (14 ml) **Streptavidin-HRP Dilution Buffer**
1 bottle (50 ml) **Wash Buffer Concentrate** 30x
1 vial (13 ml) **Substrate Solution** (tetramethyl-benzidine)
1 vial (14 ml) **Stop Solution**
1 vial (0.4 ml) **Blue-Dye**
1 vial (0.4 ml) **Green-Dye**
1 vial (0.4 ml) **Red-Dye**
4 **Adhesive Films**

5 STORAGE INSTRUCTIONS – ELISA KIT

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

6 SPECIMEN COLLECTION AND STORAGE INSTRUCTIONS

Cell culture supernatant, serum and plasma were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.
Pay attention to a possible **"Hook Effect"** due to high sample concentrations (see chapter 11). Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive monkey IL-1β. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen Hample should be brought to room temperature slowly and mixed gently. Do not thaw samples in a 37°C water bath. Do not vortex or sharply agitate samples.

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 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 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 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 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 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

Buffer concentrates should be brought to room temperature and should be diluted before starting the test procedure.

9.1 Wash Buffer (1x)

If crystals have formed in the **Wash Buffer Concentrate** (30x), warm it gently until they have completely dissolved. Pour entire contents (50 ml) of the Wash Buffer Concentrate (30x) into a clean 1500 ml graduated cylinder. Bring to final volume of 1500 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2° to 8°C. Please note that Wash Buffer is stable for 6 months. Do not use Wash Buffer if it becomes visibly contaminated during storage. Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (30x)(ml)	Distilled Water (ml)
1 - 6	25	725
1 - 12	50	1450

9.2 Streptavidin-HRP

Please note that the Streptavidin-HRP should be used within 15 minutes after dilution. Make a 1:400 dilution of the concentrated **Streptavidin-HRP** solution with Streptavidin-HRP Dilution Buffer in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Streptavidin-HRP Dilution Buffer (ml)
1 - 6	0.015	5.985
1 - 12	0.030	11.970

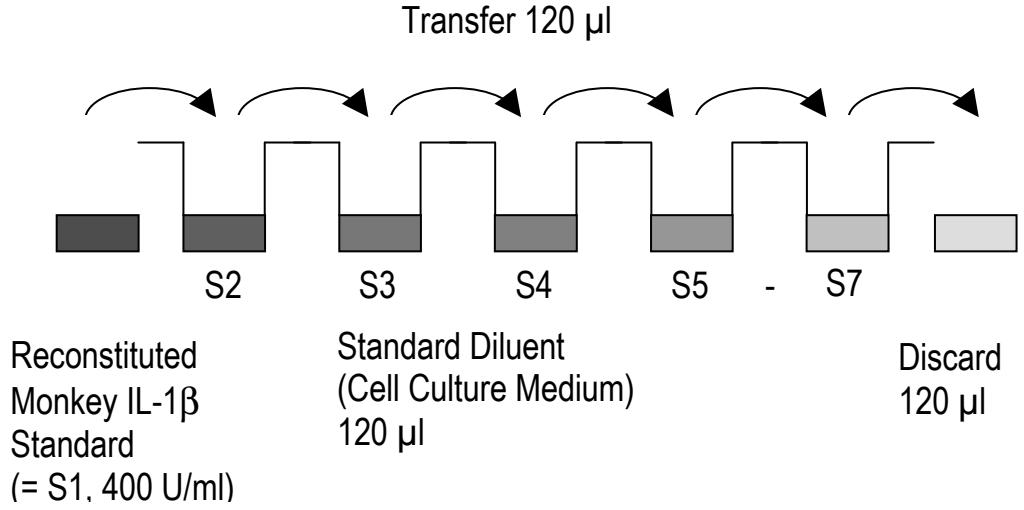
9.3 Monkey IL-1βStandard

Reconstitute **monkey IL-1β standard** by addition of distilled water.
Reconstitution volume is stated in the Quality Control Sheet. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 400 U/ml). The standard has to be used within 1 hour after reconstitution and cannot be stored.
Standard dilutions can be prepared directly on the microwell plate (see 10.b) or alternatively in tubes (see 9.3.1).

9.3.1 External Standard Dilution

Label 6 tubes, one for each standard point.
S2, S3, S4, S5, S6, S7
Then prepare 1:2 serial dilutions for the standard curve as follows:
Pipette 120 µl of Standard Diluent (for cell culture supernatant symplex use your cell culture medium instead) into tubes S2 – S7.
Pipette 120 µl of reconstituted standard (concentration = 400 U/ml) into the first tube, labelled S2, and mix (concentration of standard 2 = 200 U/ml).
Pipette 120 µl of this dilution into the second tube, labelled S3, and mix
thoroughly before the next transfer. Repeat serial dilutions 4 more times thus creating the points of the standard curve (see Figure 6).
Standard Diluent (for cell culture supernatant samples use your cell culture medium instead) serves as blank.

Figure 6



9.3 Addition of Colour-giving Reagents: Blue-Dye, Green-Dye, Red-Dye

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 standard and 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***

2. Biotin-Conjugate: Add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Biotin Conjugate.

4 ml Biotin Conjugate	40 µl ***Green-Dye***
8 ml Biotin Conjugate	80 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 Streptavidin-HRP Dilution Buffer used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the instruction booklet: Preparation of Streptavidin-HRP.

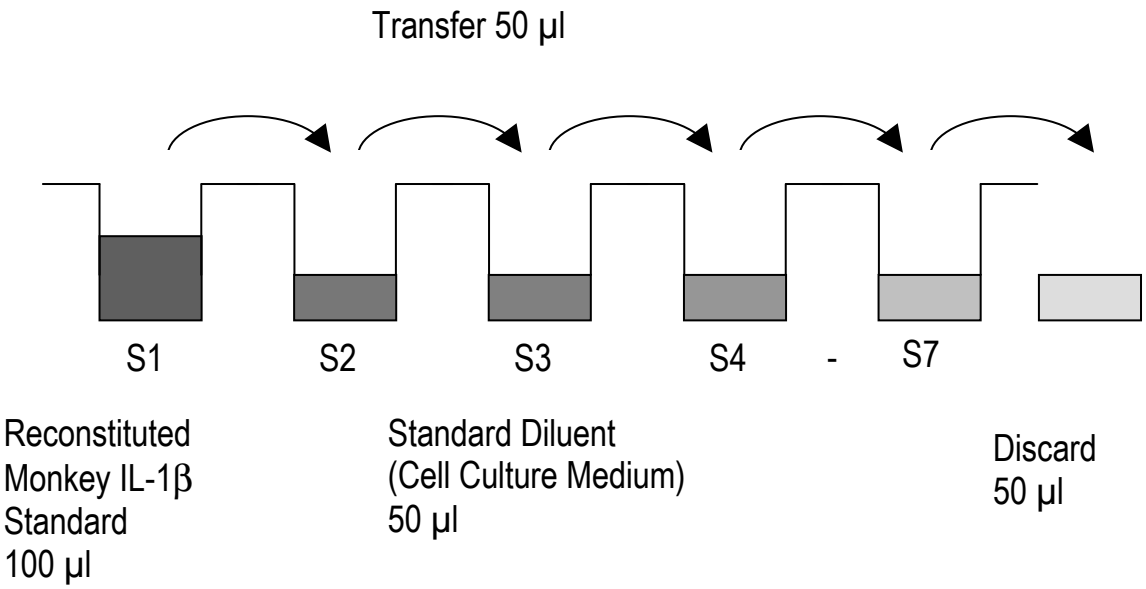
6 ml Streptavidin-HRP Dilution Buffer	24 µl ***Red-Dye***
12 ml Streptavidin-HRP Dilution Buffer	48 µl ***Red-Dye***

10 TEST PROTOCOL

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

b. **Standard dilution on the microwell plate** (Alternatively the standard dilution can be prepared in tubes - see 9.3.1): Add 50 µl of Standard Diluent (for cell culture supernatant samples use your cell culture medium instead) in duplicate to **standard wells** B1/2- G1/2, leaving A1/A2 empty. Pipette 100 µl of prepared **standard** (see Preparation of Standard 9.3, concentration = 400 U/ml) in duplicate into well A1 and A2 (see Table 1). Transfer 50 µl to wells B1 and B2. Mix the contents of wells B1 and B2 by repeated aspiration and ejection, and transfer 50 µl to wells C1 and C2, respectively (see Figure 7). Take care not to scratch the ihned surface of the microwells. Continue this procedure 4 times, creating two rows of monkey IL-1β standard dilutions ranging from 400.0 to 6.3 U/ml. Discard 50 µl of the contents from the last microwells (G1, G2) used.

Figure 7



In case of an **external standard dilution** (see 9.3.1), pipette 50 µl of these standard dilutions (S1 - S7) in the standard wells according to Table 1.

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 (400.0 U/ml)	Standard 1 (400.0 U/ml)	Sample 1	Sample 1
B	Standard 2 (200.0 U/ml)	Standard 2 (200.0 U/ml)	Sample 2	Sample 2
C	Standard 3 (100.0 U/ml)	Standard 3 (100.0 U/ml)	Sample 3	Sample 3
D	Standard 4 (50.0 U/ml)	Standard 4 (50.0 U/ml)	Sample 4	Sample 4
E	Standard 5 (25.0 U/ml)	Standard 5 (25.0 U/ml)	Sample 5	Sample 5
F	Standard 6 (12.5 U/ml)	Standard 6 (12.5 U/ml)	Sample 6	Sample 6
G	Standard 7 (6.3 U/ml)	Standard 7 (6.3 U/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

c. Add 50 µl of **Standard Diluent** (for cell culture supernatant samples use your cell culture medium instead) in duplicate to the **blank wells**.
d. Add 50 µl of each **sample** in duplicate to the **sample wells**.
e. Add 50 µl of **Biotin-Conjugate** to all wells.
f. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 3 hours.
g. Prepare **Streptavidin-HRP** (refer to Preparation of Streptavidin-HRP 9.2).
h. Remove adhesive film and empty wells. **Wash** microwell strips 3 times 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 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**.
i. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.
j. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 30 minutes.
k. Remove adhesive film and empty wells. **Wash** microwell strips 3 times according to point h. of the test protocol. Proceed immediately to the next step.
l. Pipette 100 µl of **TMB Substrate Solution** to all wells.
m. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

The colour development on the plate should be monitored and the substrate reaction stopped (see next point 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 0.60 – 0.65.

n. 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.
o. 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 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 value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the monkey IL-1β 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 monkey IL-1β 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 monkey IL-1β concentration.
- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low monkey IL-1β levels (Hook Effect). Such samples require further external predilution according to expected monkey IL-1β values with the appropriate Diluent in order to precisely quantitate the actual monkey IL-1β level.**
- It is suggested that each testing facility establishes a control sample of known monkey IL-1β concentration and runs this additional kontrol 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.

Figure 8
Representative standard curve for monkey IL-1β ELISA. Monkey IL-1β was diluted in serial 2-fold steps in Standard Diluent. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

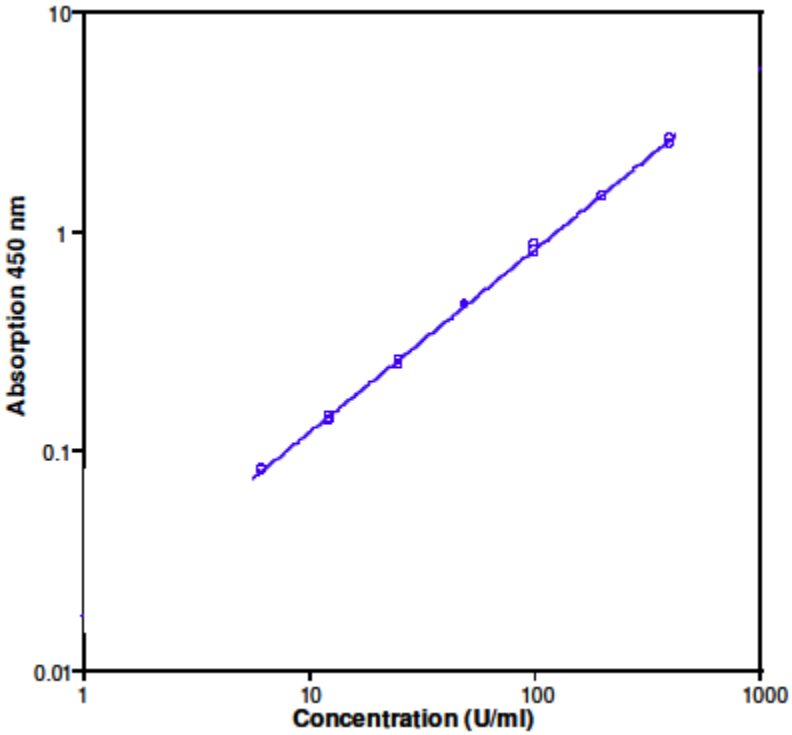


Table 2
Typical data using the monkey IL-1β ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Monkey IL-1β Concentration (U/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	400.0	2.626	2.560	2.6
	400.0	2.494		
2	200.0	1.429	1.438	0.6
	200.0	1.446		
3	100.0	0.873	0.839	4.1
	100.0	0.804		
4	50.0	0.464	0.468	0.7
	50.0	0.471		
5	25.0	0.257	0.253	1.8
	25.0	0.248		
6	12.5	0.137	0.139	1.1
	12.5	0.140		
7	6.3	0.082	0.083	0.6
	6.3	0.083		
Blank	0	0.018	0.018	
	0	0.018		

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.

13 PERFORMANCE CHARACTERISTICS

13.1 Sensitivity

The limit of detection of monkey IL-1β defined as the analyse concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.7 U/ml (mean of 6 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated from the mean of several assays per sample. The calculated overall intra-assay coefficient of variation was < 10%.

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated from the mean of the average of duplicate samples of 20 different runs performed by 3 different operators.The calculated overall inter-assay coefficient of variation was < 10%.

13.3 Spiking Recovery

The spiking recovery was evaluated by spiking monkey IL-1β into various samples. The overall mean recovery was 85%.

13.4 Dilution Linearity

Samples with different levels of monkey IL-1β were analysed at serial 2 fold dilutions with 4 replicates each. The overall mean recovery was 104%.

13.5 Specifity

The interference of circulating factors of the immune systeme was evaluated by spiking these proteins at physiologically relevantconcentrations into a monkey IL-1β positive serum. There was no crossreactivity detected.

14 REFERENCES

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15 REAGENT PREPARATION SUMMARY

15.1 Wash Buffer (1x)

Add **Wash Buffer Concentrate** 30x (50 ml) to 1500 ml distilled water.

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	25	725
1 - 12	50	1450

15.2 Streptavidin-HRP

Make a 1:400 dilution of **Streptavidin-HRP** in Streptavidin-HRP Dilution Buffer:

Number of Strips	Streptavidin-HRP (ml)	Streptavidin-HRP Dilution Buffer (ml)
1 - 6	0.015	5.985
1 - 12	0.030	11.970

15.3 Monkey IL-1β Standard

Reconstitute lyophilized **monkey IL-1β standard** with distilled water. (Reconstitution volume is stated in the Quality Control Sheet.)

16 TEST PROTOCOL SUMMARY

1. Determine the number of microwell strips required.
2. Standard dilution on the microwell plate: Add 50 µl Standard Diluent (cell culture medium for cell culture supernatant samples), in duplicate, to all standard wells leaving the first wells empty. Pipette 100 µl prepared standard into the first wells and create standard dilutions by transferring 50 µl from well to well. Discard 50 µl from the last wells. Alternatively external standard dilution in tubes (see 9.3.1): Pipette 50 µl of these standard dilutions in the microwell strips.
3. Add 50 µl Standard Diluent (cell culture medium, respectively), in duplicate, to the blank wells.
4. Add 50 µl sample in duplicate, to designated sample wells.
5. Add 50 µl Biotin-Conjugate to all wells.
6. Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C).
7. Prepare Streptavidin-HRP.
8. Empty and wash microwell strips 3 times with Wash Buffer.
9. Add 100 µl diluted Streptavidin-HRP to all wells.
10. Cover microwell strips and incubate 30 minutes at room temperature (18° to 25°C).
11. Empty and wash microwell strips 3 times with Wash Buffer.
12. Add 100 µl of TMB Substrate Solution to all wells.
13. Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
14. Add 100 µl Stop Solution to all wells.
15. Blank microwell reader and measure colour intensity at 450 nm.

NOTES

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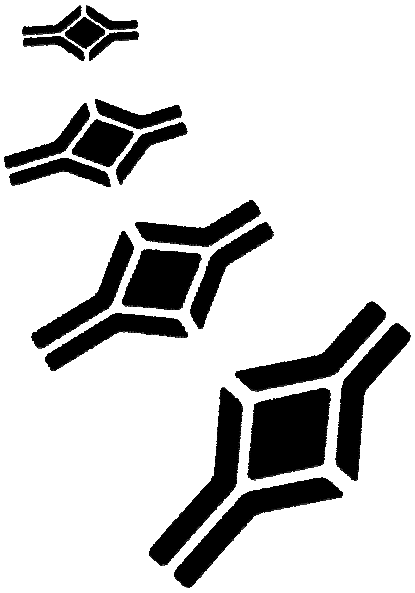

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