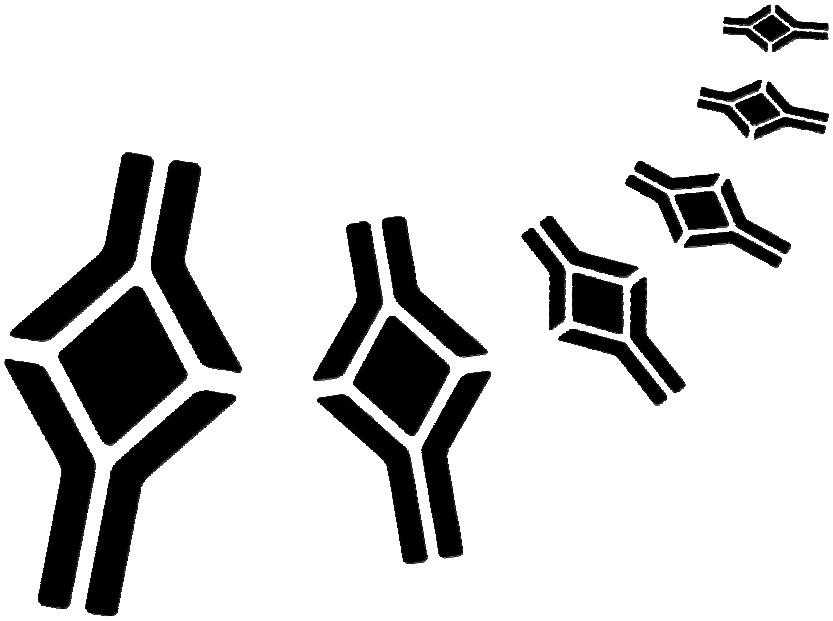
BioVendor
Research
and Diagnostic Products



MONKEY INTERLEUKIN-8 ELISA

Product Data Sheet

Cat. No.: RBMS640/3R

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-8 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of monkey IL-8. **The monkey IL-8 ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

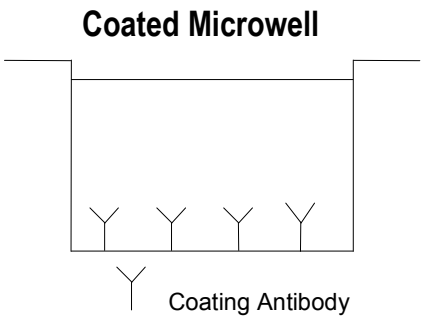
2 SUMMARY

Interleukin-8/Neutrophil-Activating Peptide-1 selectively stimulates the ability of neutrophils and T-lymphocytes to invade injured or inflamed tissue (1, 9, 11, 13). Exogenous stimuli like LPS (10), but also IL-1, TNFα and TNFβ induce the secretion of IL-8 (8) in a variety of different cell types including monocytes, endothelial and epithelial cells, peripheral blood mononuclear cells, dermal fibroblasts (8), keratinocytes (8), neutrophils (3), hepatocytes, synovial cells (4), and T-lymphocytes (11). When IL-8 was subcutaneously injected into rats, both lymphocytes and neutrophils migrated to the site of injection within 3 hours. At lower dosages, only lymphocytes migrated towards the site of injection, while at higher dosages primarily neutrophils were attracted. It was found that T-lymphocytes are 10 times more sensitive to IL-8 than neutrophils. IL-8 exerts its effects via specific cell membrane receptors (chemotactic agonist receptor-family) with homogeneous high-affinity activity and two binding sites for its ligand (2, 7). The receptor density is determined by the cell type and ranges from 300 on T-lymphocytes up to 20.000 on neutrophils (11). After binding of IL-8, the receptor expression is downregulated >90 % within 10 minutes at 37°C, together with the internalization of the ligand (11, 12). IL-8 is proteolytically degraded in the cytoplasm and released into the culture medium as soluble fragments (11). The IL-8 receptors are probably recycled (12). Besides its chemotactic influence, IL-8 exerts other distinct characteristics. In neutrophils it triggers the secretion of superoxide anions and lysosomal enzymes, thereby indirectly augmenting the permeability of blood vessels (14), and IL-8 enhances the fungicidal activity against Candida albicans. Neutrophils are more readily liberated from the bone marrow reservoir under the influence of this cytokine (11). In vitro, IL-8 stimulates a rapid Mac-1 as well as CR 1, p150,95 and LFA-1 expression on neutrophils which enables the adherence to activated vascular endothelial cells expressing e.g. ICAM-1 (5). This may account for the accumulation of neutrophils at IL-8 injection sites. Other findings suggest that endothelial-derived IL-8 may function to attenuate inflammatory events at the interface between vessel wall and blood, via inhibiting neutrophil adhesion to cytokine-activated endothelial monolayers. Therefore these cells seem to be protected against neutrophil-mediated damage (6). In basophils, besides its chemotactic effects, IL-8 stimulates the histamin liberation.
The property of IL-8 to stimulate movement of neutrophils across endothelial monolayers in vitro supports the concept of a central role for this molecule in the accumulation of neutrophils at inflammatory lesions in vivo.

3 PRINCIPLES OF THE TEST

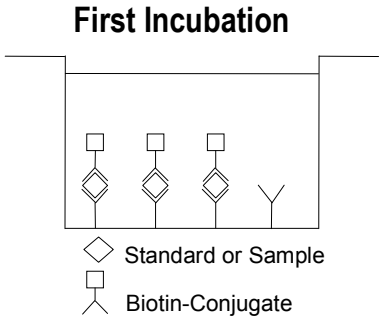
An anti-monkey IL-8 coating antibody is adsorbed onto microwells.

Figure 1



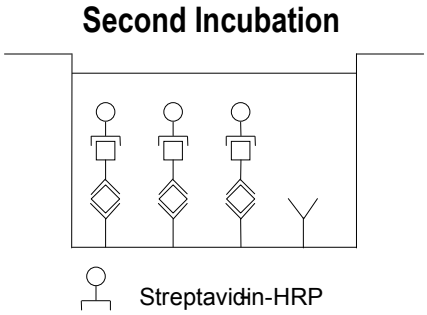
Monkey IL-8 present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-monkey IL-8 antibody is added and binds to monkey IL-8 captured by the first antibody.

Figure 2



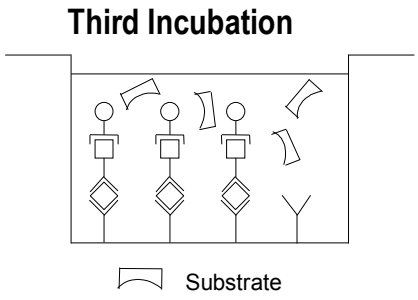
Following incubation unbound biotin-conjugated anti-monkey IL-8 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-monkey IL-8 antibody.

Figure 3



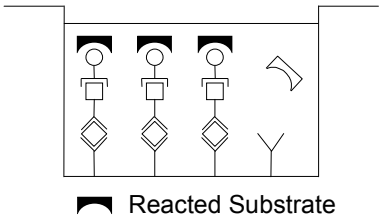
Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Figure 4



A coloured product is formed in proportion to the amount of monkey IL-8 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-8 standard dilutions and monkey IL-8 sample concentration determined.

Figure 5



4 REAGENTS PROVIDED

1 aluminium pouch with a **Antibody Coated Microtiter Strips** with monoclonal antibody to monkey IL-8
1 vial (100 µl) **Biotin-Conjugate** anti-monkey IL-8 polyclonal antibody
1 vial (150 µl) **Streptavidin-HRP**
2 vials (100 µl) monkey IL-8 **Standard Concentrate**, 125000 U/ml
1 vial (12 ml) **Sample Diluent**
1 vial (5 ml) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20 and 10% BSA)
1 bottle (50 ml) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20)
1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)
1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)
1 vial (0.4 ml) **Blue-Dye**
1 vial (0.4 ml) **Green-Dye**
1 vial (0.4 ml) **Red-Dye**
4 **Adhesive Films**

Please note: *In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the standard and Biotin-Conjugate vials. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.*

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 and serum (cynomolgus, baboon) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum from the clot as soon as possible after clotting.
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.
Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive monkey IL-8. Addition of protease inhibitors may account for better stability of samples (for sample stability refer to 13.5).
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample 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 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

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 1000 ml graduated cylinder. Bring to final volume of 1000 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.
Wash Buffer may also 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

9.2 Assay Buffer (1x)

Pour the entire contents (5 ml) of the **Assay Buffer Concentrate** (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently to avoid foaming.
Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.
Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

9.3 Biotin-Conjugate

Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.
Make a 1:100 dilution of the concentrated **Biotin-Conjugate** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

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

Please note: *In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the Biotin-Conjugate vials. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.*

9.4 Streptavidin-HRP

Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.
Make a 1:200 dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (1x) (ml)
1 - 6	0.03	5.97
1 - 12	0.06	11.94

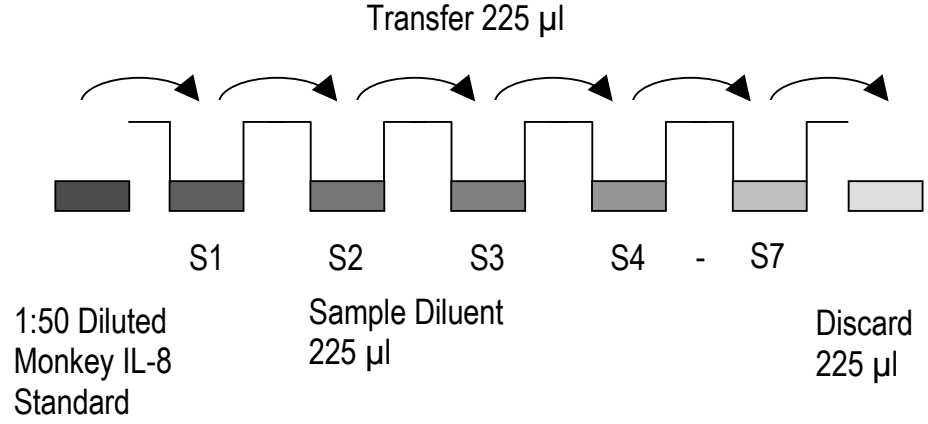
9.5 Monkey IL-8 Standard

The concentrated **monkey IL-8 standard** must be diluted 1:50 with Assay Buffer (1x) just prior to use in a clean plastic test tube according to the following dilution scheme:
20 µl concentrated **monkey IL-8 standard** + 980 µl Assay Buffer (1x). Shake gently to mix (concentration of diluted standard = 2500 U/ml).
Please note: *In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the standard vials. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.*
Standard dilutions can be prepared directly on the microwell plate (see 10.c) or alternatively in tubes (see 9.5.1).

9.5.1 External Standard Dilution

Label 7 tubes, one for each standard point.
S1, S2, S3, S4, S5, S6, S7
Then prepare 1:2 serial dilutions for the standard curve as follows:
Pipette 225 µl of Sample Diluent into each tube.
Pipette 225 µl of diluted standard (concentration = 2500 U/ml) into the first tube, labelled S1, and mix (concentration of standard 1 = 1250 U/ml). Pipette 225 µl of this dilution into the second tube, labelled S2, and mix thoroughly before the next transfer. Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 6).
Sample Diluent serves as blank.

Figure 6



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

2. Biotin-Conjugate: Before dilution of the concentrated Biotin-Conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet: Preparation of Biotin-Conjugate.

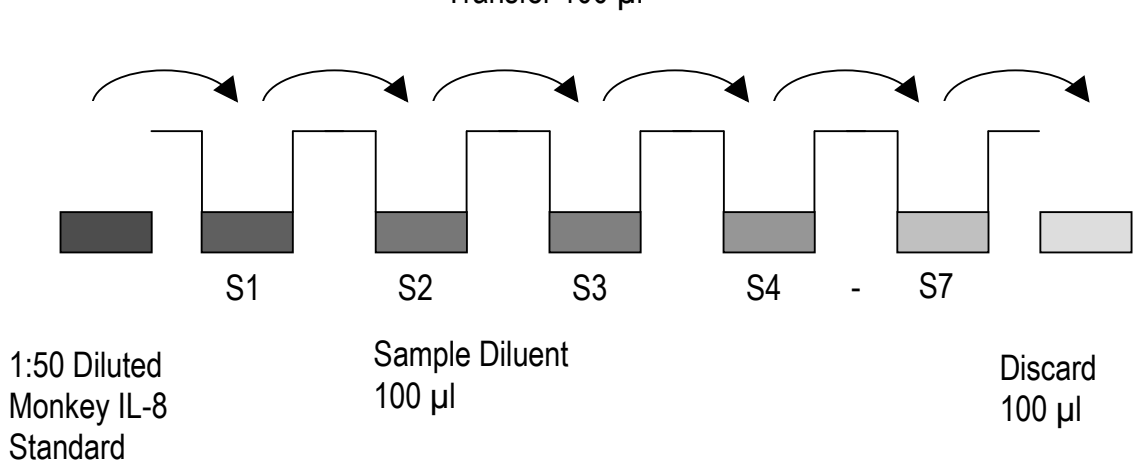
3 ml Assay Buffer (1x)	30 µl ***Green-Dye***
6 ml Assay Buffer (1x)	60 µl ***Green-Dye***

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 Assay Buffer (1x) used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the instruction booklet: Preparation of Streptavidin-HRP.

6 ml Assay Buffer (1x)	24 µl ***Red-Dye***
12 ml Assay Buffer (1x)	48 µl ***Red-Dye***

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. Wash the microwell strips twice with approximately 400 µl **Wash** Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. **Do not allow wells to dry**.
c. **Standard dilution on the microwell plate** (Alternatively the standard dilution can be prepared in tubes - see 9.5.1): Add 100 µl of Sample Diluent in duplicate to all **standard wells**. Pipette 100 µl of prepared **standard** (see Preparation of Standard 9.5, concentration = 2500 U/ml) in duplicate into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 1250 U/ml), and transfer 100 µl to wells B1 and B2, respectively (see
d. Figure 7). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of monkey IL-8 standard dilutions ranging from 1250 to 19 U/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 7



In case of an **external standard dilution** (see 9.5.1), pipette 100 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 (1250 U/ml)	Standard 1 (1250 U/ml)	Sample 1	Sample 1
B	Standard 2 (625 U/ml)	Standard 2 (625 U/ml)	Sample 2	Sample 2
C	Standard 3 (313 U/ml)	Standard 3 (313 U/ml)	Sample 3	Sample 3
D	Standard 4 (156 U/ml)	Standard 4 (156 U/ml)	Sample 4	Sample 4
E	Standard 5 (78 U/ml)	Standard 5 (78 U/ml)	Sample 5	Sample 5
F	Standard 6 (39 U/ml)	Standard 6 (39 U/ml)	Sample 6	Sample 6
G	Standard 7 (19 U/ml)	Standard 7 (19 U/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

e. Add 100 µl of **Sample Diluent** in duplicate to the **blank wells**.
f. Add 50 µl of **Sample Diluent** to the **sample wells**.
g. Add 50 µl of each **sample** in duplicate to the **sample wells**.
h. Prepare **Biotin-Conjugate** (see Preparation of Biotin-Conjugate 9.3).
i. Add 50 µl of **Biotin-Conjugate** to all wells.
j. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours, if available on a microplate shaker set at 100 rpm.
k. Prepare **Streptavidin-HRP** (refer to Preparation of Streptavidin-HRP 9.4).
l. Remove adhesive film and empty wells. **Wash** microwell strips 3 times according to point b. of the test protocol. Proceed immediately to the next step.
m. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.
n. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 1 hour, if available on a microplate shaker set at 100 rpm.
o. Remove adhesive film and empty wells. **Wash** microwell strips 3 times according to point b. of the test protocol. Proceed immediately to the next step.
p. Pipette 100 µl of **TMB Substrate Solution** to all wells.
q. 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 0.6 – 0.65.

r. 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.
s. 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-8 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-8 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-8 concentration.
- **If instructions in this protocol have been followed samples have been diluted 1:2 (50 µl sample + 50 µl Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 2).**
- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low monkey IL-8 levels (Hook Effect). Such samples require further external predilution according to expected monkey IL-8 values with Sample Diluent in order to precisely quantitate the actual monkey IL-8 level.**
- It is suggested that each testing facility establishes a control sample of known monkey IL-8 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 8. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

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

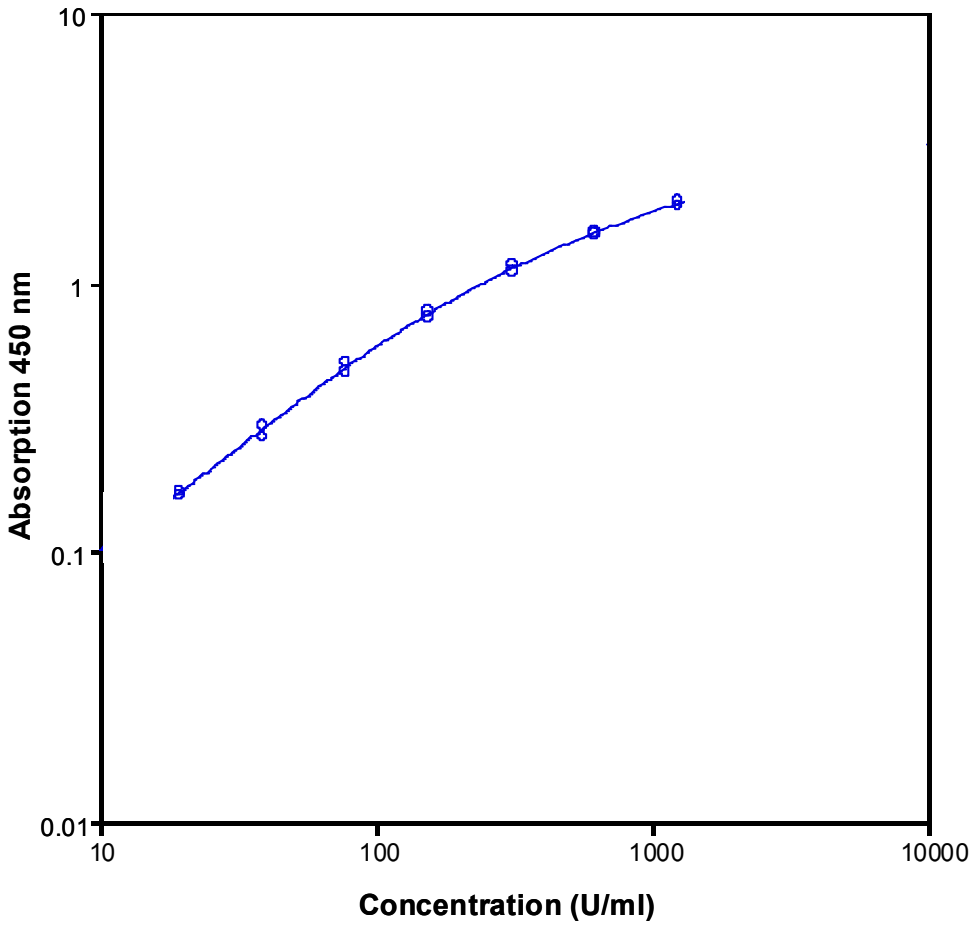


Table 2
Typical data using the monkey IL-8 ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Monkey IL-8 Concentration (U/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	1250	2.003	1.967	1.8
	1250	1.931		
2	625	1.522	1.513	0.6
	625	1.504		
3	313	1.175	1.132	3.8
	313	1.088		
4	156	0.787	0.769	2.3
	156	0.751		
5	78	0.507	0.490	3.6
	78	0.472		
6	39	0.292	0.279	4.7
	39	0.266		
7	19	0.167	0.165	1.2
	19	0.163		
Blank	0	0.038	0.040	
	0	0.042		

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 mouse IgG antibodies or mouse IgG antibody coupled molecules in the course of animal experiments has significantly increased the number of animals with anti-mouse IgG antibodies (equivalent to human anti-mouse IgG antibodies - HAMA). These anti-mouse IgG antibodies 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 monkey IL-8 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 3 U/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 8 serum samples containing different concentrations of monkey IL-8. 2 standard curves were run on each plate. Data below show the mean monkey IL-8 concentration and the coefficient of variation for each sample (see

Table 3). The calculated overall intra-assay coefficient of variation was 6.3%.

Table 3
The mean monkey IL-8 concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Monkey IL-8 Concentration (U/ml)	Coefficient of Variation (%)
1	1	928.5	7
	2	890.0	7
	3	769.1	8
2	1	435.9	4
	2	441.4	8
	3	480.4	3
3	1	230.6	5
	2	229.9	5
	3	234.6	6
4	1	100.0	7
	2	82.3	8
	3	93.3	8
5	1	55.4	4
	2	43.3	10
	3	52.1	7
6	1	650.5	4
	2	597.4	6
	3	768.9	5
7	1	341.3	4
	2	314.0	3
	3	364.8	11
8	1	395.0	9
	2	391.6	6
	3	325.0	9

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of monkey IL-8. 2 standard curves were run on each plate. Data below show the mean monkey IL-8 concentration and the coefficient of variation calculated on 18 determinations of each sample (see

Table 4). The calculated overall inter-assay coefficient of variation was 8.7%.

Table 4
The mean monkey IL-8 concentration and the coefficient of variation of each sample

Sample	Mean Monkey IL-8 Concentration (U/ml)	Coefficient of Variation (%)
1	862.5	9.6
2	452.5	5.4
3	231.8	1.1
4	91.9	9.7
5	50.3	12.5
6	672.3	13.1
7	340.0	7.5
8	370.5	10.7

13.3 Spiking Recovery

The spiking recovery was evaluated by spiking 4 levels of monkey IL-8 into serum samples. Recoveries were determined in 3 independent experiments with 6 replicates each.
The amount of endogenous monkey IL-8 in unspiked serum was substracted from the spike values.
The recovery ranged from 72% to 125% with an overall mean recovery of 88%.

13.4 Dilution Linearity

4 serum samples with different levels of monkey IL-8 were analysed at serial 2 fold dilutions with 4 replicates each.
The recovery ranged from 90% to 119% with an overall recovery of 107% (see Table 5).

Table 5

Sample	Dilution	Expected Monkey IL-8 Concentration (U/ml)	Observed Monkey IL-8 Concentration (U/ml)	Recovery of Expected Monkey IL-8 Concentration (%)
1	1:2	--	964.6	--
	1:4	482.4	503.0	104
	1:8	251.5	272.3	108
	1:16	136.1	130.4	96
2	1:2	--	462.4	--
	1:4	231.3	242.5	105
	1:8	121.3	128.3	106
	1:16	64.1	76.4	119
3	1:2	--	62.1	--
	1:4	31.0	28.1	90
	1:8	14.0	14.5	104
	1:16	7.3	8.3	114
4	1:2	--	394.9	--
	1:4	197.5	219.5	111
	1:8	109.8	124.4	113
	1:16	62.3	73.8	119

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the monkey IL-8 levels determined. There was no significant loss of monkey IL-8 immunoreactivity detected 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 monkey IL-8 level determined after 24 h. There was no significant loss of monkey IL-8 immunoreactivity detected during storage under above conditions.

14 REFERENCES

1) Baggiolini M., A. Walz, and S.L. Kunkel. (1989). Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J. Clin. Invest. 84, 1045-1049.
2) Baldwin, E.T., I.T. Weber, R.S. Charles, J.-C. Xuan, E. Appella, M. Yamada, K. Matsushima, B.F.P. Edwards, G.M. Clore, A.M. Groneborn, and A. Wlodawer. (1991). Crystal structure of interleukin 8: Symbiosis of NMR and crystallography. Proc. Natl. Acad. Sci. USA (Biochemistry) 88, 502-506.
3) Bazzoni, F., M.A. Cassatella, F. Rossi, M. Ceska, B. Dewald, and M. Baggiolini. (1991). Phagycytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/interleukin 8. J. Exp. Med. 173, 771-774.
4) DeMarco D., S.L. Kunkel, R.M. Strieter, M. Basha, and R.B. Zurier. (1991). Interleukin-1 induced gene expression of neutrophil activating protein (interleukin-8) and monocyte chemotactic peptide in human synovial cells. Biochem. Biophys. Res. Comm. 174, 411-416.
5) Detmers, P.A., S.K. Lo, E. Olsen-Egbert, R. Walz, M. Baggiolini, and Z.A. Cohn. (1990). Neutrophil-activating protein 1/interleukin 8 stimulates the binding activity of the leukocyte adhesion receptor CD11b/CD 18 on human neutrophils. J. Exp. Med. 171, 1155-1162.
6) Gimbrone, M.A., M.S. Obin, A.F. Brock, E.A. Luis, P.E. Haas, C.A. Hebert, Y.K. Yip, D.W. Leung, D.G. Lowe, W.J. Kohr, W.C. Darbonne, K.B. Bechtol, and J.B. Baker. (1989). Endothelial interleukin-8: A novel inhibitor of leukocyte-endothelial interactions. Science 246, 1601-1603.
7) Grob, P.M., E. David, T.C. Warren, R.P. De Leon, P.R. Farina, and C.A. Homon. (1990). Characterization of a receptor for human monocyte-derived neutrophil chemotactic factor/interleukin-8. J. Biol. Chem. 265, 8311-8316.
8) Larsen, C.G., A.O. Anderson, J.J. Oppenheim, and K. Matsushima. (1989). Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumor necrosis factor. Immunology 68, 31-36.
9) Leonard, E.J. (1990). NAP-1 (IL-8). Immunol. Today 11, 223-224.
10) Martich, G.D., R.L. Danner, M. Ceska, and A.F. Suffredini. (1991). Detection of interleukin 8 and tumor necrosis factor in normal humans after intravenous endotoxin: The effect of antiinflammatory agents. J. Exp. Med. 173, 1021-1024.
11) Matsushima, K. and J.J. Oppenheim. (1989). Interleukin 8 and MCAF: Novel inflammatory cytokines inducible by IL-1 and TNF. Cytokine 1, 2-13.
12) Samanta, A.K., J.J. Oppenheim, and K. Matsushima. (1990). Interleukin 8 (monocyte-derived neutrophil chemotactic factor) dynamically regulates its own receptor expression

on human neutrophils. J. Biol. Chem. 265, 183-189.
13) Smith, W.B., J.R. Gamble, I. Clark-Lewis, and M.A. Vadas. (1991). Interleukin-8 induces neutrophil transendothelial migration. Immunology 72, 65-72.
14) Sticherling, M., E. Bornscheuer, J.-M. Schröder, and E. Christophers. (1991). Localization of neutrophil-activating peptide-1/interleukin-8 immunoreactivity in normal and psoriatic skin. J. Invest. Dermatol. 96, 26-30.

15 REAGENT PREPARATION SUMMARY

15.1 Wash Buffer

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

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

15.2 Assay Buffer (1x)

Add **Assay Buffer Concentrate** 20x (5 ml) to 95 ml distilled water.

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

15.3 Biotin-Conjugate

Make a 1:100 dilution of **Biotin-Conjugate** in Assay Buffer (1x):

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

15.4 Streptavidin-HRP

Make a 1:200 dilution of **Streptavidin-HRP** in Assay Buffer (1x):

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (1x) (ml)
1 - 6	0.03	5.97
1 - 12	0.06	11.94

15.5 Monkey IL-8 Standard

The concentrated **monkey IL-8 standard** must be diluted 1:50 with Assay Buffer (1x).

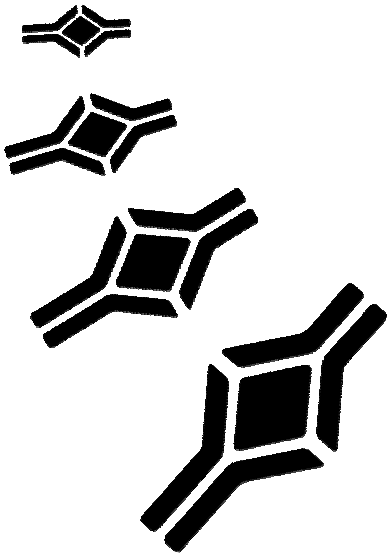
16 TEST PROTOCOL SUMMARY

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100 µl Sample Diluent, in duplicate, to all standard wells. Pipette 100 µl prepared standard into the first wells and create standard dilutions by transferring 100 µl from well to well. Discard 100 µl from the last wells. Alternatively external standard dilution in tubes (see 9.5.1): Pipette 100 µl of these standard dilutions in the microwell strips.
4. Add 100 µl Sample Diluent, in duplicate, to the blank wells.
5. Add 50 µl Sample Diluent to sample wells.
6. Add 50 µl sample in duplicate, to designated sample wells.
7. Prepare Biotin-Conjugate.
8. Add 50 µl Biotin-Conjugate to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C).
10. Prepare Streptavidin-HRP.
11. Empty and wash microwell strips 3 times with Wash Buffer.
12. Add 100 µl diluted Streptavidin-HRP to all wells.
13. Cover microwell strips and incubate 1 hour at room temperature (18º to 25ºC).
14. Empty and wash microwell strips 3 times with Wash Buffer.
15. Add 100 µl of TMB Substrate Solution to all wells.
16. Incubate the microwell strips for about 10 minutes at room temperature (18°to 25°C).
17. Add 100 µl Stop Solution to all wells.
18. Blank microwell reader and measure colour intensity at 450 nm.

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

NOTES

BioVendor
Research
and Diagnostic Products



HEADQUARTERS: **BioVendor Laboratorní medicína, a.s.**	**CTPark Modrice Evropska 873**	**664 42 Modrice CZECH REPUBLIC**	**Phone: +420-549-124-185 Fax: +420-549-211-460**	**E-mail:info@biovendor.com Web:www.biovendor.com**
EUROPEAN UNION: BioVendor GmbH	Im Neuenheimer Feld 583	D-69120 Heidelberg GERMANY	Phone: +49-6221-433-9100 Fax: +49-6221-433-9111	E-mail: infoEU@biovendor.com
USA, CANADA AND MEXICO: BioVendor LLC	1463 Sand Hill Road Suite 227	Candler, NC 28715 USA	Phone: +1-828-670-7807 +1-800-404-7807 Fax: +1-828-670-7809	E-mail: infoUSA@biovendor.com
CHINA - Hong Kong Office: BioVendor Laboratories Ltd	Room 4008 Hong Kong Plaza, No.188	Connaught Road West Hong Kong, CHINA	Phone: +852-2803-0523 Fax: +852-2803-0525	E-mail: infoHK@biovendor.com
CHINA – Mainland Office: BioVendor Laboratories Ltd	Room 2405 YiYa Tower TianYu Garden, No.150	Lihe Zhong Road Guang Zhou, CHINA	Phone: +86-20-8706-3029 Fax: +86-20-8706-3016	E-mail: infoCN@biovendor.com