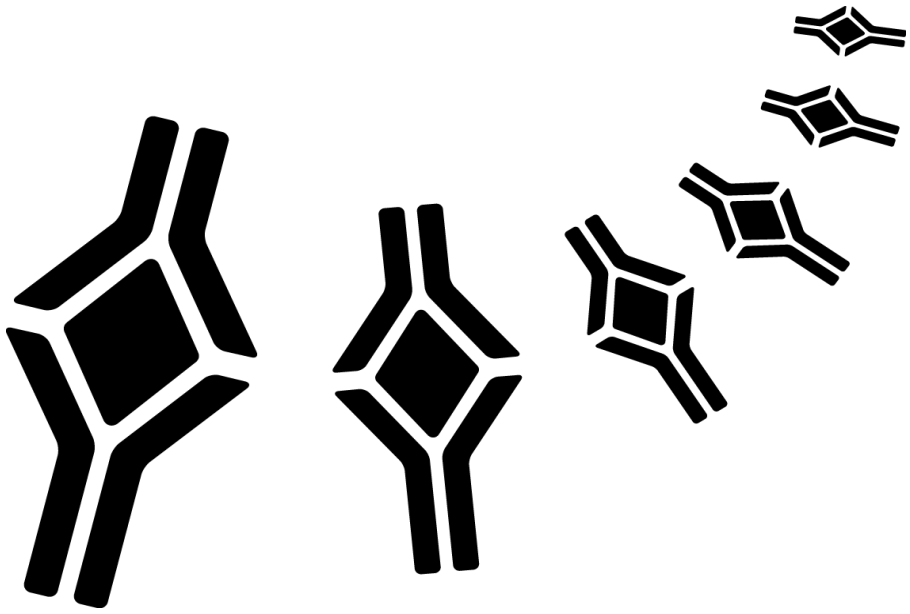
BioVendor
Research
and Diagnostic Products



PORCINE TNF-APLHA ELISA

Product Data Sheet

Cat. No.: RA19018R

For Research Use Only

CONTENTS

1. PORCINE TNF-ALPHA ELISA 3
2. PRECAUTION FOR USE 3
3. BACKROUND 4
4. PRINCIPLE OF THE ASSAY 4
5. MATERIAL REQUIRED BUT NOT PROVIDED 6
6. SAMPLE COLLECTION AND PREPARATION 6
7. REAGENT PREPARATION 6
8. ASSAY PROCEDURE 8
9. DATA ANALYSIS 11
10. ACCEPTABLE RANGE 11
11. TYPICAL RESULTS 12
12. ASSAY VALIDATION AND CHARACTERISTICS 13
13. ASSAY TROUBLE SHOOTING 13
14. BIBLIOGRAPHY 14

NOTES 15

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

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

1. PORCINE TNF-ALPHA ELISA

96 wells
Storage: 4°C
Expiry date: stated on the package

This kit contains:

REAGENTS	**Quantity**	**Form**
Antibody Coated Microtiter Strips (TNF-apha precoated 96-well strip plate)	1	
Conjugate Solution (Streptavidin Poly-HRP Tracer)	1	liquid
Biotin-labelled Antibody	1	liquid
Porcine TNF-alpha Standard	2	lyophilized
Dilution Buffer (EIA buffer)	1	lyophilized
Wash Solution	1	liquid
HRP Substrate Solution	1	liquid
HRP Stop Solutin	1	liquid
Tween 20	1	liquid
Cover Sheet	1	

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 37 samples in duplicate.

2. PRECAUTION FOR USE

Users are recommended to read all instructions for use before starting work.

Each time a new pipet tip is used, aspirate a sample or reagent and dispense it back into the same vessel.
Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only.
- Not for diagnostic use.
- Do not pipet liquids by mouth.
- Do not use kit components beyond the expiration date.
- Do not eat, drink or smoke in area in which kit reagents are handled.
- Avoid splashing.

HRP Stop Solution and HRP Substrate Solution are harmful solution. To avoid any contact, wear eye, hand, face and clothing protection when handling these.

Wearing gloves, laboratory coat and glasses is recommended when assaying kit material and samples.

Temperature
Unless otherwise specified, all the experiments are done at room temperature (RT), than is around +20°C. Working at +25°C or more affects the assay.

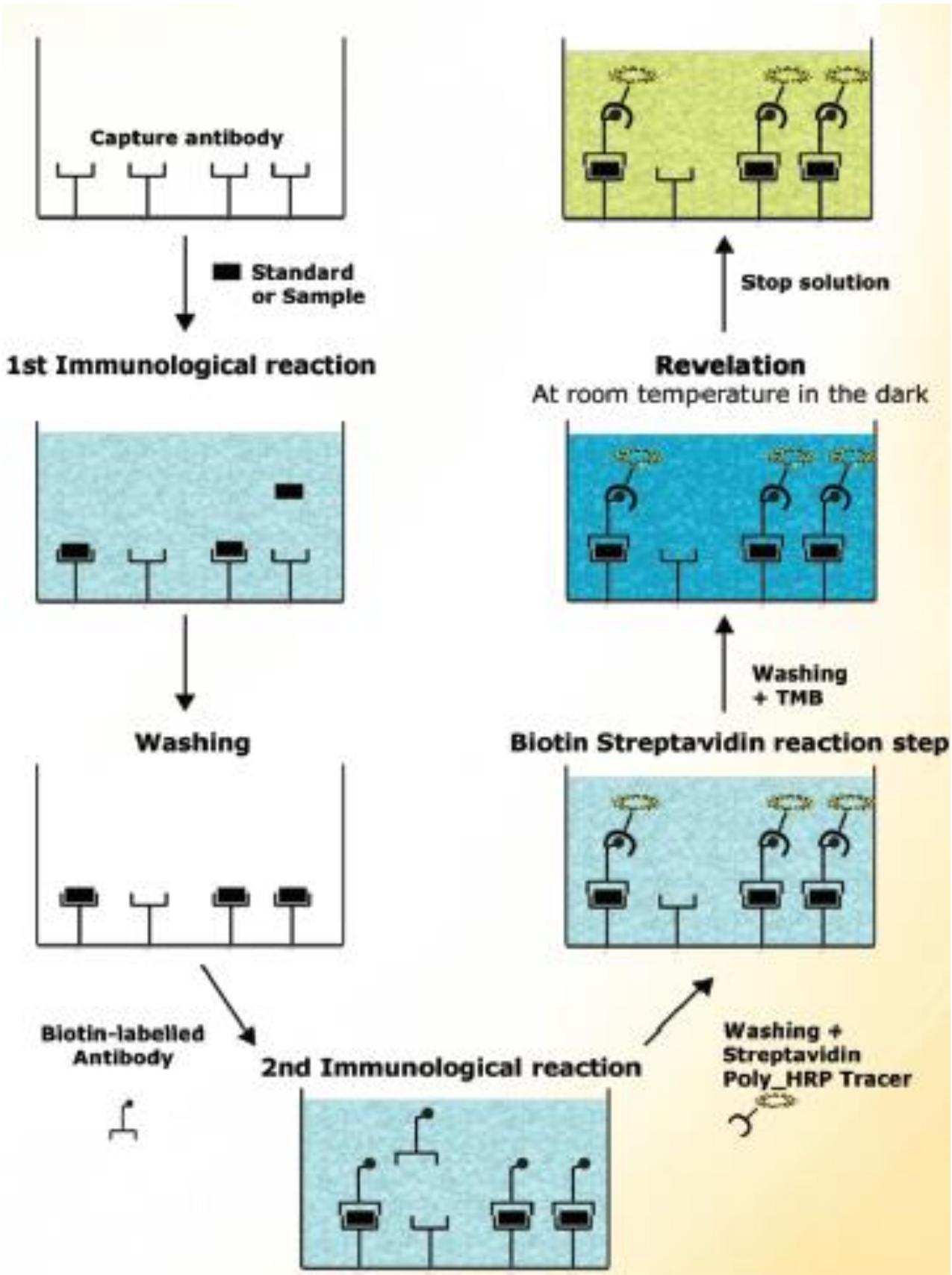
3. BACKROUND

TNF-alpha
Known as cachectin, TNF-alpha (Tumor Necrosis Factor-alpha) is a glycoprotein of 156 amino acids produced from a precursor processed by action of a metalloprotease. Isolated for the first time in 1975 by Carswell [1], it's a cytokine of the innate immunity.
Interleukin-1 or bacterial endotoxins stimulate the production of TNF-alpha by immune cells [2] (activated macrophages, NK cells, monocytes) and non-immune cells [3, 4, 5] (keratinocytes, vascular smooth muscle cells and granulosa lutein cells).
The main function of TNF-alpha is to act as an immunomodulator and as a mediator of inflamatory responses in the phenomena of host resistance to infection.
It allows neutrophil and macrophage recruitment to infectious sites by stimulating the production of adhesion molecules and chemokines (chemotactic cytokines) by endothelial cells. TNFalpha also acts on the hypothalamus to trigger the fever [6].
Over-production of TNF-alpha has been implicated in a number of pathological conditions including cachexia, septic shock and autoimmune disorders [7, 8, 9, 10].

4. PRINCIPLE OF THE ASSAY

This Enzyme Immunometric Assay (EIA/ELISA) is based on a sandwich technique. The wells of the plate supplied are coated with a polyclonal antibody specific to TNF-alpha (porcine).
TNF-alpha (porcine) introduced into the wells (standard or sample) will be bound by the polyclonal antibody coated on the plate and is then detected by a second polyclonal antibody tagged with
biotin also specific for TNF-alpha (porcine).
The two antibodies then form a sandwich by binding on different parts of the TNF-alpha (porcine).
The sandwich is immobilised on the plate so reagents in excess may be washed away.
The immunological complex is revealed by the interaction between biotin and streptavidin labelled with HRP (Tracer).
The concentration of TNF-alpha (porcine) is determined by measuring the enzymatic activity of immobilized Tracer using TMB. The Tracer acts on TMB to form a yellow compound after the reaction has been stopped.
The intensity of the colour, which is determined by spectrophotometry at 450 nm, is

proportional to the amount of TNF-alpha (porcine) present in the well during the immunological incubation.
The principle of the assay is summarised below:



5. MATERIAL REQUIRED BUT NOT PROVIDED

In addition to standard laboratory equipment, the following material is required:

- Precision micropipettes (20 to 1000 µL)
- Spectrophotometer plate reader (450 nm filter)
- Microplate washer (or wash-bottles)
- Orbital microplate shaker
- Multichannel pipette and disposable tips 30-300 µL
- Ultra pure water (cat. number S0001)
- Polypropylene tubes

Water used to prepare all EIA reagents and buffers must be Ultra Pure, deionized & free from organic contaminants traces.
Do not use distilled water, HPLC-grade water or sterile water.

- Ulta pure water may be purchased from BioVendor (cat. number S0001)

6. SAMPLE COLLECTION AND PREPARATION

This assay may be used to measure TNF-alpha (porcine).
It is the responsibility of the user to check the compatibility of the assay with the study matrix.

General precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection or should be stored at -20°C or at -80°C

7. REAGENT PREPARATION

All reagents need to be brought to room temperature (around +20°C) prior to the assay.

- **Dilution Buffer**

Reconstitute Dilution Buffer with 25 mL of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.
Stability at 4°C: 1 month.
Before use, filter the Buffer on 0,22 µm filter.

- **TNF-alpha (porcine) Standard**

Reconstitute the TNF-alpha (porcine) Standard vial with 1 mL of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

The concentration of the fi rst standard (S1) is 2 ng/mL. Prepare seven polypropylene tubes (for the seven other standards) and add 500 µL of Dilution Buffer into each tube. Then prepare the standard concentrations by serial dilutions as follow:

Standard	Volume of Standard	Volume of Dilution Buffer	Standard concentration ng/mL
S1	-	-	2.00
S2	500 µL of S1	500 µL	1.00
S3	500 µL of S2	500 µL	0.50
S4	500 µL of S3	500 µL	0.25
S5	500 µL of S4	500 µL	0.13
S6	500 µL of S5	500 µL	0.06
S7	500 µL of S6	500 µL	0.03
S8	500 µL of S7	500 µL	0.02

Stability at +4°C: within the day.

- **Biotin-labelled Antibody**

The TNF-alpha Biotin-labelled Antibody is supplied concentrated 10 times. Calculate the volume needed (number of wells multiplied by 0.1 mL) and then dilute the TNF-alpha Biotin-labelled Antibody solution with the appropriate volume of Dilution Buffer.

Example: for 40 wells you need 4 mL of TNF-alpha biotin labelled Antibody (40 x 0.1 mL), add 0.4 mL of TNF-alpha Biotin-labelled Antibody in 3.6 mL of Dilution Buffer.
Stability of diluted antibody at +4°C: within the day.

- **Wash Buffer**

Dilute 2 mL of concentrated Wash Buffer with 800 mL of UltraPure water. Add 400 µL of Tween 20.
Use a magnetic stirring bar to mix the content.
Stability at +4°C: 1 month.

8. ASSAY PROCEDURE

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

Plate preparation
Prepare the Wash Buffer as indicated in the reagent preparation section.
Open the plate packet and select the suffi cient strips for your assay. Put unused strips back in the zip lock bag with the dessicant pocket and properly close it.
Stability at +4°C: 1 month.
Rinse each well 5 times with Wash Buffer (300 µL/well).
Just before distributing the reagents and samples, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Plate set-up
A plate set-up is suggested on this page. The contents of each well may be recorded on the sheet provided with the kit.

Pipetting the reagents
All samples and reagents must reach room temperature prior to performing the assay.
Use different tips to pipet all the reagents.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bk	S7	S3	*	*	*	*	*	*	*	*	*
B	Bk	S7	S3	*	*	*	*	*	*	*	*	*
C	Bk	S6	S2	*	*	*	*	*	*	*	*	*
D	NSB	S6	S2	*	*	*	*	*	*	*	*	*
E	NSB	S5	S1	*	*	*	*	*	*	*	*	*
F	NSB	S5	S1	*	*	*	*	*	*	*	*	*
G	S8	S4	*	*	*	*	*	*	*	*	*	*
H	S8	S4	*	*	*	*	*	*	*	*	*	*

Bk : Blank
S1-S8: Standards 1-8
* : Samples
NSB : Non Specific Binding
QC: Quality Controls

Before pipetting, equilibrate the pipette tips in each reagent.
Do not touch the liquid already in the well when expelling with the pipette tip.

- **Dilution Buffer**

Dispense 100 µL to Non Specific Binding (NSB).

- **Porcine TNF-alpha Standards/Sample**

Dispense 100 µL of each of the eight standards (S1 to S8) in duplicate to appropriate wells.
Start with the lowest concentration standard (S8) and equilibrate the tip in the next higher standard before pipetting.

Incubating the plate
Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm.

Washing the plate
Rinse each well 5 times with the Wash Buffer (300 µL/well). Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Pipetting the reagents
- **Biotin-labelled Antibody**

Dispense 100 µL to each well, except Blank (Bk) wells.

Incubating the plate
Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm.

Washing the plate
Rinse each well 5 times with the Wash Buffer (300 µL/well). Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Pipetting the reagents
- **Conjugate Solution (Streptavidin Poly-HRP Tracer)**

Dispense 100 µL to each well, except Blank (Bk) wells.

Incubating the plate
Cover the plate with the cover sheet and incubate 30 minutes at room temperature, shaking at 300 rpm.

Developing and reading the plate
- Rinse each well 5 times with 300 µL of Wash Buffer. At the end of the last washing step, empty the plate and blot the plate on a paper towel to discard any trace of liquid.
- Add 100 µL of HRP Substrate Solution to each well.
- Incubate the plate in the dark at room temperature without shaking. For the time, look at the lot specific Quality Control Sheet. In general, revelation time is 10 min.
- Add 100 µL of HRP Stop Solution to each well.

• Wipe the bottom of the plate with a paper towel, and make sure that no liquid has splashed outside the wells.
• Read the plate at 450 nm (yellow color).

Easy Sampling Enzyme Immunoassay Protocole (volumes are in µL)				
	Blank	NSB	Standard	Sample
Dilution Buffer	-	100	-	-
Standard	-	-	100	-
Sample	-	-	-	100
Cover plate, incubate 60 minutes at room temperature under orbital shaking at 300 rpm.				
Wash strips 5 times with 300 µL/well Discard liquid from the well and dry on absorbent paper				
Biotin-labelled Antibody	-	100		
Cover plate, incubate 60 minutes at room temperature under orbital shaking at 300 rpm.				
Wash strips 5 times with 300 µL/well Discard liquid from the well and dry on absorbent paper				
Conjugate Solution	-	100		
Cover plate, incubate 60 minutes at room temperature under orbital shaking at 300 rpm.				
Wash strips 5 times with 300 µL/well Discard liquid from the well and dry on absorbent paper				
HRP Substrate Solution	100			
Incubate the plate in the dard wihtout agitation				
HRP Stop Solution	100			
Read the plate at 450 nm				

9. DATA ANALYSIS

Make sure that your plate reader has subtracted the absorbance readings of the blank wells (absorbance of HRP Substrate Solution alone) from the absorbance readings of the rest of the plate.If it is not the case, please do it.

- Calculate the average absorbance for each NSB, standards and samples.
- For each standard, plot the absorbance on y axis versus the concentration on x axis. Draw a best-fit line through the points.
- To determine the concentration of your samples, find the absorbance value of each sample on the y axis.
- Read the corresponding value on the x axis which is the concentration of your unknown sample.
- Samples with a concentration greater than 2 ng/mL should be re-assayed after dilution in Dilution Buffer.
- Most plate readers are supplied with curve-fitting software capable of graphing this type of data (logit/log or 4-parameter). If you have this type of software, we recommend using it. Refer to it for further information.

10. ACCEPTABLE RANGE

- NSB absorbance: ≤ 0.060 A.U.
- Limit of detection: ≤ 0.02 ng/ml

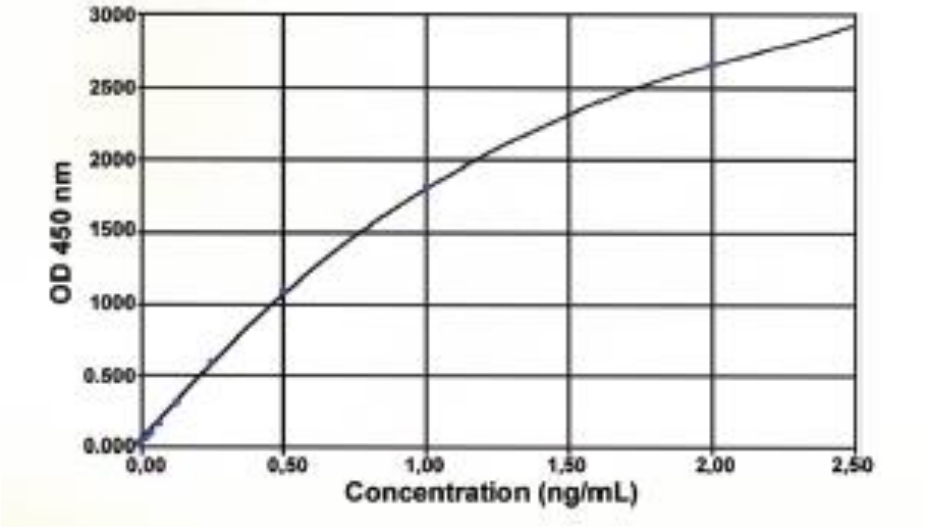
11. TYPICAL RESULTS

Example data

The following data are for demonstration purpose only. Your data may be different and still correct.
These data were obtained using all reagents as supplied in this kit under the following conditions: 10 minutes developing at room temperature, reading at 450 nm. A 4 parameter logistic fitting was used to determine the concentrations.

Standard	**Porcine TNF-alpha ng/ml**	**Absorbance (A.U.)**
Standard S1	2.00	2.645
Standard S2	1.00	1.803
Standard S3	0.50	1.082
Standard S4	0.25	0.592
Standard S5	0.13	0.318
Standard S6	0.06	0.175
Standard S7	0.03	0.104
Standard S8	0.02	0.066
NSB	0.00	0.030

Porcine TNF-alpha Standard Curve



12. ASSAY VALIDATION AND CHARACTERISTICS

Cross-reactivity:

Species	Cross-reactivity
Recombinant TNF-alpha (bovine)	Strong
Recombinant TNF-alpha (canine)	Strong
Recombinant TNF-alpha (dolphin)	Strong
Recombinant TNF-alpha (equine)	None
Recombinant TNF-alpha (feline)	Strong
Recombinant TNF-alpha (guinea porcine)	None

Limit of detection:
Ccalculated as the concentration of TNF-alpha (porcine) corresponding to the NSB average plus
three standard deviation is ≤0.02 ng/mL.

13. ASSAY TROUBLE SHOOTING

Absorbance values too low:
- one reagent has not been dispensed
- incorrect preparation
- assay performed before reagents reached room temperature
- reading time not long enough

High signal and background in all wells:
- inefficient washing
- overdeveloping (incubation time should be reduced)
- high ambient temperature

High dispersion of duplicates:
- poor pipetting technique
- irregular plate washing

14. BIBLIOGRAPHY

- Carswell E. A., Old L. J., Kassel R. L. et *al*

An endotoxin-induced serum factor that causes necrosis of tumors.
Proc Natl Acad Sci USA (1975) 72:3666-70.

- Trebichavský I., Splíchal I., Barot-Ciorbaru R.

Expression of TNF-alpha in pig fetal cells stimulated *in vitro*.
Folia Microbiol (Praha).1995;40(4):417-20.

- Allen D.G., Riviere J.E., Monteiro-Riviere N.A.

Cytokine induction as a measure of cutaneous toxicity in primary and immortalized porcine keratinocytes exposed to jet fuels, and their relationship to normal human epidermal keratinocytes.
Toxicol Lett. 2001 Mar 8;119(3):209-17.

- Newman W.H., Zhang L.M., Lee D.H. et *al*

Release of tumor necrosis factor-alpha from coronary smooth muscle: activation of NF-kappaB and inhibition by elevated cyclic AMP.
J Surg Res. 1998 Dec;80(2):129-35

- Vézina S. A., Roberge D., Fournier M. et *al*

Cloning of porcine cytokine-specifi c cDNAs and detection of porcine tumor necrosis factor alpha, interleukin 6 (IL-6), and IL-1 beta gene expression by reverse transcription PCR and chemiluminescence hybridization.
Clin Diagn Lab Immunol. 1995 Nov;2(6):665-71

- Sundgren-Andersson A.K., Ostlund P., Bartfai T.

IL-6 is essential in TNF-alpha-induced fever.
Am J Physiol. 1998 Dec;275(6 Pt 2):R2028-34.

- Vilcek J., Lee T.H.

Tumor necrosis factor. New insights into the molecular mechanisms of its multiple actions
J Biol Chem 1991 Apr 25;266(12):7313-6.

- Ware C.F. et *al*

Apoptosis mediated by the TNF-related cytokine and receptor families.
J. Cellular Biochemistry 1996 60:47.

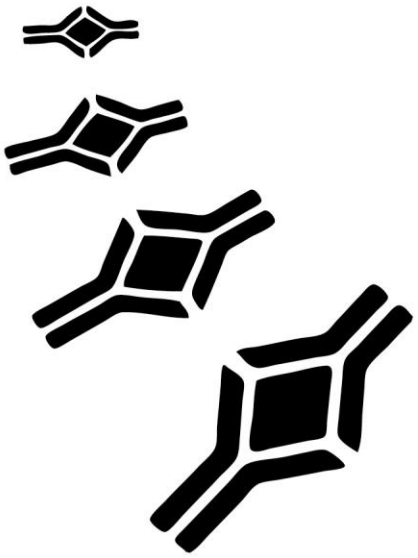
- Aggarwal B.B., Vilcek J., eds

Tumor Necrosis Factor: Structure, Function and Mechanism of Action.
Marcel Dekker, 1991 Inc., New York

- Beutler B., Cerami A.

The biology of cachectin/TNF-a primary mediator of the host response.
Annu Rev Immunol 1989;7:625-55.

NOTES



BioVendor – Laboratorní medicína a.s.
Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420-549-124-185, Fax: +420-549-211-460
E-mail: info@biovendor.com, sales@biovendor.com
Web: www.biovendor.com

There are BioVendor branches and distributors near you.
To find the office closest to you, visit **www.biovendor.com/contact**

BioVendor
Research
and Diagnostic Products