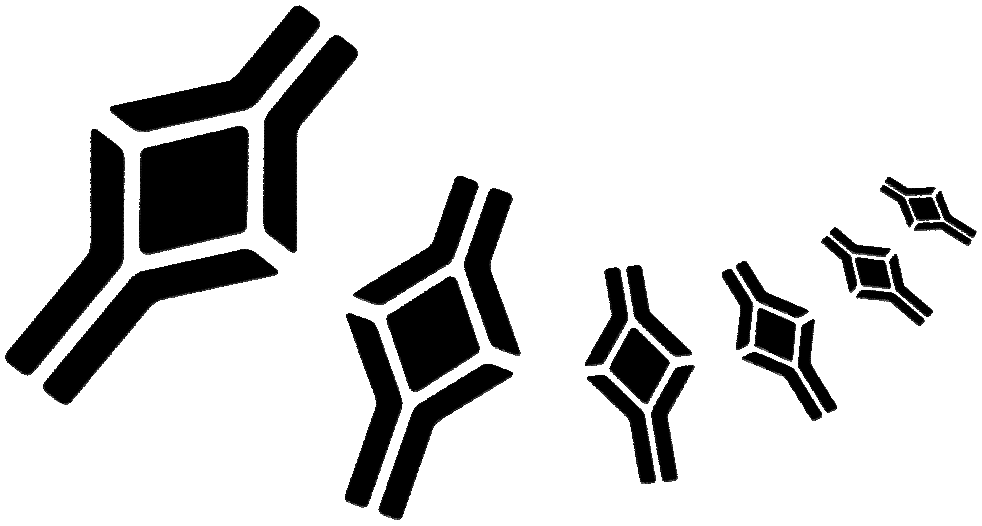
BioVendor
Research
and Diagnostic Products



Human sAPO- 1/Fas ELISA

Product Data Sheet

Cat. No.: RBMS245R

For Research Use Only

VERSION 51 080708

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 sAPO-1/Fas ELISA is an enzyme-linked immunosorbent assay for quantitative detection of soluble human APO-1/Fas in cell culture supernatants, human serum, plasma or other body fluids. **The sAPO-1/Fas ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2 SUMMARY

Programmed cell death or apoptosis is the most common form of eukaryotic cell death and is found during tumor regression and embryonic development. In the process of selection and eliminiation of autoreactive B and T cells apoptosis is an important process and therefore a prerequisite for the homeostasis of the immune system. Apoptosis is characterized by changes in cellular morphology (e.g. nuclear condensation, membrane bledding) and biochemically by rapid induction on DNA fragmentation.

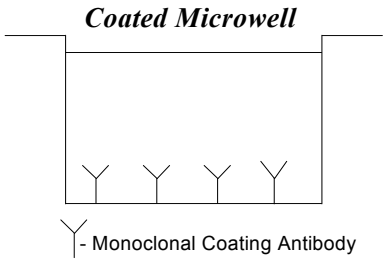
APO-1/Fas (CD95), a member of the TNF/NGF receptor superfamily, is a glycosylated 48kD surface protein containing a single transmembrane region [4, 10, 14]. APO-1 is expressed on a variety of human B and T cell lines, on many different tumor cells [3, 7, 9] and on various normal human tissues [8, 12]. Triggering of APO-1 by its ligand or by certain anti-APO-1 monoclonal antibodies results in rapid induction of programmed cell death, apoptosis, in susceptible cells [11, 12]. The tissue distribution of APO-1 and of the APO-1 ligand suggests that the APO-1 receptor/ligand system plays an important role in various aspects of mammalian development and especially in the homeostasis of the immune system [11].

Expression of the APO-1 cell surface protein is enhanced by IFN-γ and TNF and by activation in lymphocytes [2, 4, 5, 13, 14]. APO-1 also occurs in a soluble form (sAPO-1) devoid of a transmembrane region [1,.

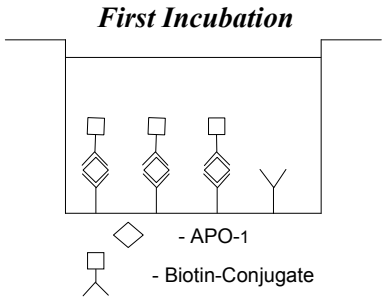
Elevated sAPO-1 levels have been reported in sera from patients with high-and low-grade malignant B- and T-cell leukemias [6] and systemic lupus erythematosus (Knipping et al., submitted). sAPO-1 may prevent cells from undergoing APO-1 ligand induced apoptosis [1]. Hence, secretion of sAPO-1 may provide a mechanism for tumor cells to excape immunosurveillance and may be involved in leukemogenesis.

3 PRINCIPLES OF THE TEST

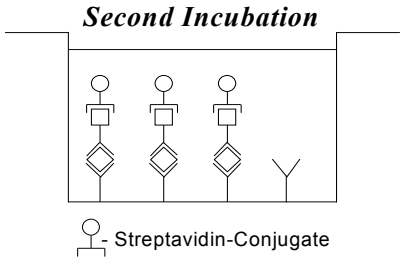
An anti-sAPO-1/Fas monoclonal coating antibody is adsorbed onto microwells.

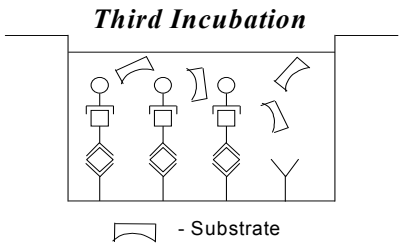


sAPO-1/Fas present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated monoclonal anti-sAPO-1/Fas is added and binds to sAPO-1/Fas captured by the first antibody.

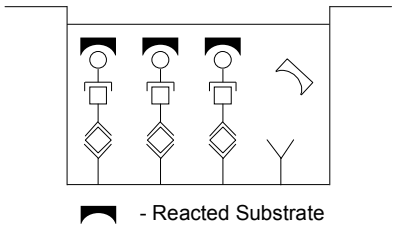


Following incubation unbound biotin conjugated anti-sAPO-1/Fas is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-sAPO-1/Fas. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.





A coloured product is formed in proportion to the amount of sAPO-1/Fas 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 sAPO-1/Fas standard dilutions and sAPO-1/Fas sample concentration determined.



4 REAGENTS PROVIDED

1 aluminium pouch with **a Antibody Coated Microtiter Strips** with Monoclonal Antibody (murine) to human sAPO-1/Fas

1 vial (100 µl) **Biotin-Conjugate anti-sAPO-1/Fas** monoclonal (murine) antibody

1 vial (150 µl) **Streptavidin-HRP**

2 vials **sAPO-1/Fas Standard,** lyophilized; 2000 pg/ml upon reconstitution

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

1 vial (5 ml) **Assay Buffer Concentrate** 20 x (PBS with 1 % Tween 20 and 10 % BSA)

1 bottle (12 ml) **Sample Diluent**

1 vial (15 ml) **Substrate Solution**

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

2 vials (0.4 ml each), **Green-Dye, Red-Dye**

4 **adhesive Plate Covers**

Reagent Labels

5 STORAGE INSTRUCTIONS

Store kit reagents between 2° and 8°C as indicated. 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.
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°C and separated rapidly before storing at -20°C to avoid loss of bioactive sAPO-1/Fas. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. 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.

- 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 reagents.

- Exposure to acids will inactivate 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 if 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

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°C. 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. Assay Buffer

Mix the contents of the bottle well. Add contents of **Assay Buffer Concentrate** (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days. Assay Buffer may 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

C. Preparation of Biotin-Conjugate

Make a 1:100 dilution with **Assay Buffer** (reagent B) in a clean plastic tube as needed according to the following table:

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

D. Preparation of sAPO-1/Fas Standard

Reconstitute **sAPO-1/Fas Standard** by addition of distilled water. Refer to the Certificate of Analysis for current volume of Distilled water needed for reconstitution of standard.. Shake or swirl gently to ensure solubilisation. Store reconstituted Standard promptly at - 20°C after use.

E. Preparation of Streptavidin-HRP

Make a 1:240 dilution with Assay Buffer (reagent B) of the concentrated **Streptavidin-HRP** solution as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (ml)
1 - 6	0.025	6.0
1 - 12	0.050	12.0

F. Addition of colour-giving reagents: 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 (***Green-Dye, Red-Dye***) can be added to the reagents according to the following guidelines:

1. Biotin-Conjugate: Before dilution of the concentrated conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer 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	30 µl ***Green-Dye***
6 ml Assay Buffer	60 µl ***Green-Dye***
12 ml Assay Buffer	120 µl ***Green-Dye***

2. 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 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	24 µl ***Red-Dye***
12 ml Assay Buffer	48 µl ***Red-Dye***

10 TEST PROTOCOL

a. Mix all reagents thoroughly without foaming before use.

b. 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 sAPO-1/Fas from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

c. 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.

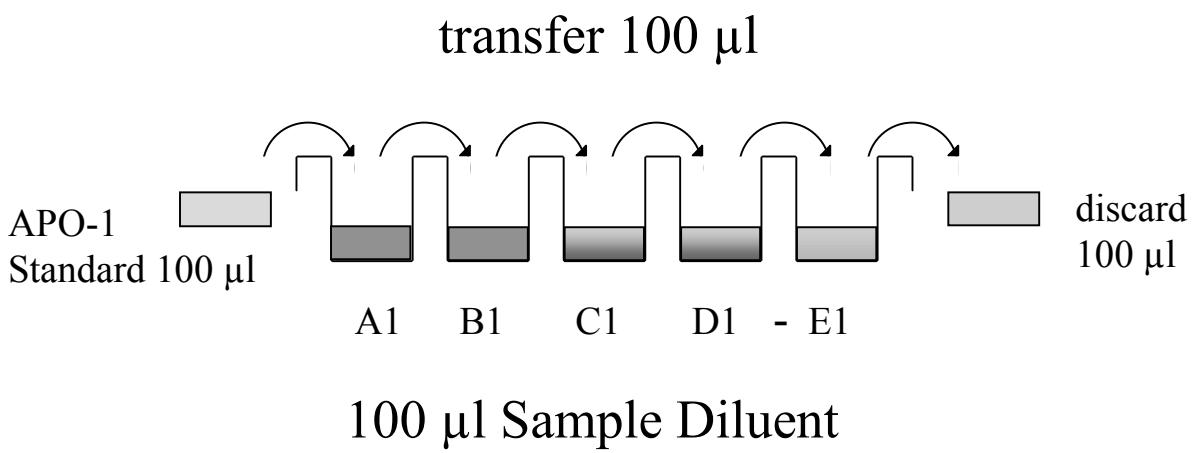
d. Add samples to the microwell plate according to the following scheme:

Figure 1. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (1000 pg/ml)	Standard 1 (1000 pg/ml)	Sample 1	Sample 1
B	Standard 2 (500 pg/ml)	Standard 2 (500 pg/ml)	Sample 2	Sample 2
C	Standard 3 (250 pg/ml)	Standard 3 (250 pg/ml)	Sample 3	Sample 3
D	Standard 4 (125 pg/ml)	Standard 4 (125 pg/ml)	Sample 4	Sample 4
E	Standard 5 (63 pg/ml)	Standard 5 (63 pg/ml)	Sample 5	Sample 5
F	Standard 6 (32 pg/ml)	Standard 6 (32 pg/ml)	Sample 6	Sample 6
G	Standard 7 (16 pg/ml)	Standard 7 (16 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

e. Start by adding 100 µl of **Sample Diluent** in duplicate to all standard wells and to the blank wells.
f. Add 90 µl of **Sample Diluent** to all wells designated for samples.
g. Add 10 µl of each **Sample**, in duplicate, to the designated wells and mix contents.
h. Prepare standard dilutions by pipetting 100 µl of diluted (Refer to preparation of reagents, 9.D.) **sAPO-1/Fas 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 sAPO-1/Fas standard dilutions ranging from 1000 to 16 pg/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 2. Preparation of sAPO-1/Fas standard dilutions:



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

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

k. Cover with a **Plate Cover** and incubate at 37°C for 1 hour.

l. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

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

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

o. Cover with a **Plate Cover** and incubate at 37°C for 1 hour.

p. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

q. Pipette 100 µl of **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. 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 sAPO-1/FAS standards.

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 sAPO-1/Fas concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating sAPO-1/Fas 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 sAPO-1/Fas concentration.

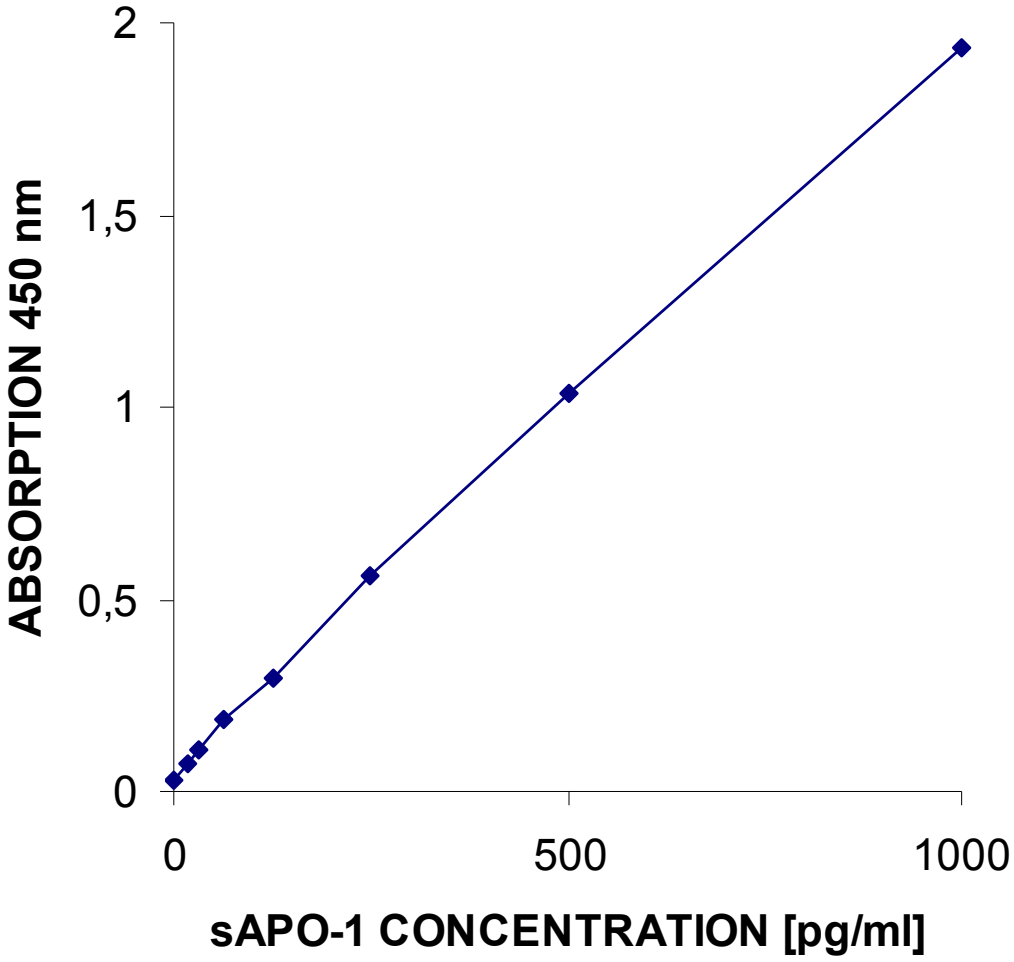
- **For samples which have been diluted according to the instructions given in this manual 1:10, the concentration read from the standard curve must be multiplied by the dilution factor (x10).**

- **Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sAPO-1/Fas levels. Such samples require further dilution with Sample Diluent in order to precisely quantitate the actual sAPO-1/Fas level.**

- It is suggested that each testing facility establishes a control sample of known sAPO-1/Fas 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 sAPO-1/Fas ELISA. sAPO-1/Fas 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 sAPO-1/Fas ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	sAPO-1/Fas Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	1000	1.915	1.937	1.6
	1000	1.958		
2	500	0.981	1.037	7.6
	500	1.092		
3	250	0.564	0.562	0.8
	250	0.558		
4	125	0.296	0.297	0.2
	125	0.297		
5	63	0.176	0.186	6.9
	63	0.194		
6	32	0.103	0.111	9.6
	32	0.118		
7	16	0.072	0.075	4.7
	16	0.077		
Blank	0	0.028	0.028	
	0	0.027		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. temperature effects). Furthermore shelf life of the kit may effect 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. 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.

- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibody (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the Sample Diluent.

13 PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection of sAPO-1/Fas 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 13.2 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 8 serum samples containing different concentrations of sAPO-1/Fas. Two standard curves were run on each plate. Data below show the mean sAPO-1/Fas concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 4.5 %.

Positive Sample	Experiment	sAPO-1/Fas Concentration (pg/ml)	Coefficient of Variation (%)
1	1	2336	1.0
	2	2210	9.7
	3	2256	14.6
2	1	2109	5.5
	2	2207	8.7
	3	2135	3.3
3	1	1727	1.7
	2	1841	7.1
	3	1682	3.3
4	1	1703	6.0
	2	1855	3.6
	3	1767	5.1
5	1	3442	3.0
	2	3653	0.5
	3	3570	4.5
6	1	1422	2.1
	2	1430	4.1
	3	1357	1.3
7	1	2066	4.3
	2	2248	1.7
	3	2095	1.7
8	1	1366	4.2
	2	1353	7.7
	3	1351	4.6

b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by two technicians. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sAPO-1/Fas. Two standard curves were run on each plate. Data below show the mean sAPO-1/Fas 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 3.1 %.

Sample	sAPO-1/Fas Concentration (pg/ml)	Coefficient of Variation (%)
1	2267	2.8
2	2150	2.4
3	1750	4.7
4	1775	4.3
5	3555	3.0
6	1403	2.8
7	2136	4.6
8	1357	0.6

C. Spiking Recovery

The spiking recovery was evaluated by spiking four levels of sAPO-1/Fas into normal human serum. As shown below recoveries were determined in three independent experiments with 6 replicates each. The amount of endogenous sAPO-1/Fas in unspiked serum was subtracted from the two spike values. Recoveries ranged from 86 to 118 % with an overall mean recovery of 100 %.

sAPO-1/Fas Spike (pg/ml)	Experiment	Recovery % sAPO-1/Fas
6000	1	86
	2	98
	3	92
3000	1	89
	2	105
	3	99
1500	1	87
	2	118
	3	113
750	1	102
	2	112
	3	95

D. Dilution Linearity

Four serum samples with different levels of sAPO-1/Fas 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 87 % to 115 % with an overall mean recovery of 102 %.

Sample	Dilution	sAPO-1/Fas Concentration (pg/ml) Expected Value	Observed Value	% Recovery of Exp. Value
1	1:10	--	2134	--
	1:20	1067	1158	109
	1:40	533	579	109
	1:80	267	306	115
2	1:10	--	1894	--
	1:20	947	847	90
	1:40	473	428	90
	1:80	237	258	109
3	1:10	--	1653	--
	1:20	826	887	107
	1:40	413	441	107
	1:80	207	216	105
4	1:10	--	1706	--
	1:20	853	738	87
	1:40	426	399	94
	1:80	213	212	100

E. Sample Stability

a. Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored frozen at -20°C and thawed up to 5 times, and sAPO-1/Fas levels determined. There was no significant loss of sAPO-1/Fas by freezing and thawing up to 5 cycles of freezing and thawing.

b. 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 sAPO-1/Fas level determined after 24 h. There was no significant loss of sAPO-1/Fas immunoreactivity during storage at above conditions.

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. sAPO-1/Fas 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 used in one study.

G. Specificity

The interference of circulating factors of the immune system was evaluated by spiking several of these proteins at physiologically relevant concentrations into a sAPO-1/Fas positive serum. There was no detectable cross reactivity.

H. Expected Values

A panel of 8 sera from apparently healthy blood donors (males and females) was tested for sAPO-1/Fas. The detected sAPO-1/Fas levels ranged between 1334 and 2411 pg/ml with a mean level of 1609 pg/ml.

14 REFERENCES

1) Chen, J., Zhou, T., Liu, C., Shapiro, J.P., Brauer, M.J., Kiefer, M.C., Barr, P.J. and Mountz, J.D. (1994) Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule.
   Science, 263, 1759-1762.
2) Daniel, P.T. and Krammer, P.H. (1994) Activation induces sensitivity toward APO-1 (CD95)-mediated apoptosis in human B cells.
   J. Immunol.,152, 5624-5632.
3) Debatin, K.M., Goldmann, C.K., Bamford, R., Waldmann, T.A. and Krammer, P.H. (1990) Monoclonal-antibody-mediated apoptosis in adult T-cell leukaemia.
   Lancet, 335, 497-500
4) Itoh, N., Yonehara, S., Ishii, A., Yonehara, M., Mizushima, S., Sameshima, M., Hase, A., Seto, Y. and Nagata S. (1991) The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis.
   Cell, 66, 233-243.
5) Klas, C., Debatin, K.M., Jonker, R.R. and Krammer, P.H. (1993) Activation interferes with the APO-1 pathway in mature human T cells.
   Int. Immunol., 5, 625-630.
6) Knipping, E., Debatin, K.M., Stricker, K., Heilig, B., Eder, A. and Krammer, P.H. (1995) Identification of soluble APO-1 in supernatants of human B- and T-cell lines and increased serum levels in B- and T-cell leukemias.
   Blood, 85, 1562-1569.
7) Leithauser, F., Dhein, J., Mechtersheimer, G., Koretz, K., Bruderlein, S., Henne, C., Schmidt, A., Debatin, K.M., Krammer, P.H. and Moller, P. (1993) Constitutive and induced expression of APO-1, a new member of the nerve growth factor/tumor necrosis factor receptor superfamily, in normal and neoplastic cells.
   Lab. Invest., 69, 415-429.

8) Moller, P., Henne, C., Leithauser, F., Eichelmann, A., Schmidt, A., Bruderlein, S., Dhein, J. and Krammer, P.H. (1993) Coregulation of the APO-1 antigen with intercellular adhesion molecule-1 (CD54) in tonsillar B cells and coordinate expression in follicular center B cells and in follicle center and mediastinal B-cell lymphomas.
Blood, 81, 2067-2075.
9) Munker, R., Lubbert, M., Yonehara, S., Tuchnitz, A., Mertelsmann, R. and Wilmanns, W. (1995) Expression of the Fas antigen on primary human leukemia cells.
Ann. Hematol., 70, 15-17.
10) Oehm, A., Behrmann, I., Falk, W., Pawlita, M., Maier, G., Klas, C., Li Weber, M., Richards, S., Dhein, J., Trauth, B.C. and et al (1992) Purification and molecular cloning of the APO-1 cell surface antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily. Sequence identity with the Fas antigen.
J.Biol. Chem., 267, 10709-10715.
11) Suda, T., Takahashi, T., Golstein, P. and Nagata, S. (1993) Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family.
Cell, 75, 1169-1178.
12) Trauth, B.C., Klas, C., Peters, A.M., Matzku, S., Moller, P., Falk, W., Debatin, K.M. and Krammer, P.H. (1989) Monoclonal antibody-mediated tumor regression by induction of apoptosis.
Science, 245, 301-305.
13) Watanabe Fukunaga, R., Brannan, C.I., Copeland, N.G., Jenkins, N.A. and Nagata, S. (1992) Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis.
Nature, 356, 314-317.
14) Yonehara, S., Ishii, A. and Yonehara, M (1989) A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen co-downregulated with the receptor of tumor necrosis factor.
J. Exp. Med., 169, 1747-1756.

15 REAGENT PREPARATION SUMMARY

A. Wash Buffer

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

B. Assay Buffer

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

C. Biotin-Conjugate

Make a 1:100 dilution according to the table.

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

D. Standard

Reconstitute **sAPO-1/Fas Standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial.

E. Streptavidin-HRP

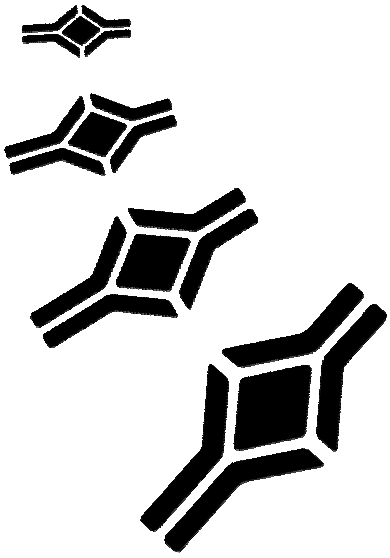
Number of Strips	Streptavidin-HRP (ml)	Assay-Buffer (ml)
1 - 6	0.025	6.0
1 - 12	0.050	12.0

16 TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Sample Diluent**, in duplicate, to all standard wells
- Add 100 µl **Sample Diluent**, in duplicate, to the blank wells
- Add 90 µl **Sample Diluent**, in duplicate, to sample wells
- Add 10 µl **Sample**, in duplicate, to designated wells
- Pipette 100 µl diluted **sAPO-1/Fas Standard** into the first wells and create standard dilutions ranging from 1000 to 16 pg/ml by transferring 100 µl from well to well. Discard 100 µl from the last wells
- Prepare **Biotin-Conjugate**
- Add 50 µl of diluted **Biotin-Conjugate** to all wells
- Cover microwell strips and incubate 1 hour at 37°C
- Prepare **Streptavidin-HRP solution**
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of diluted **Streptavidin-HRP** to all wells
- Cover microwell strips and incubate 1 hour at 37°C
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for about 15 minutes at room temperature (18°to 25°C).
- Add 100 µl **Stop Solution** to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

Note: For samples which have been diluted according to the instructions given in this manual 1:10, the concentration read from the standard curve must be muliplied by the dilution factor (x10). Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sAPO-1/Fas levels. Such samples require further dilution with Sample Diluent in order to precisely quantitate the actual sAPO-1/Fas level.

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