

Read the package insert carefully before using the product

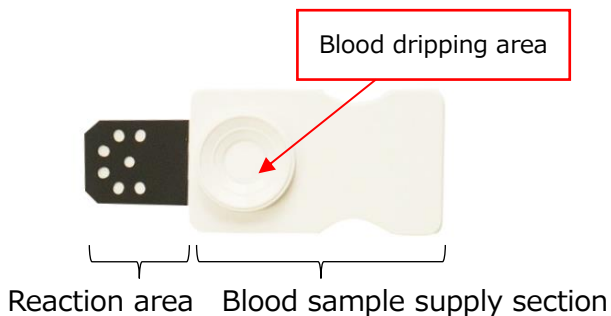
BoviLab Downer Cow Slide 3 (AST, Ca, CK, IP, Mg, NEFA)
for BoviLab Blood analyzer

[General precautions]

1. Not for human use.
2. This product is a reagent used for bovine blood component analysis. Do not use it for any other purpose.
3. Comprehensively judge the condition based on the analytical finding of this product as well as the results and symptoms of other related analysis.
4. We do not guarantee any method other than that described in the package insert, or its usage for purposes other than the intended one.
5. This product is a dedicated reagent for BoviLab Blood analyzer. Read the Quick Start Guide and the User Guide carefully before using the device.

[Shape, structure etc. (kit configuration)]

This product is a slide type and consists of a blood sample supply section and a reaction section.



(Components involved in the reaction system)

BoviLab AST	· Sodium L (+)-aspartate monohydrate
BoviLab Ca	· Arsenazo III (2,2'-(1,8-dihydroxy-3,6-disulfonaphthylene-2,7-bisazo) bisbenzenearsonic acid, 2,7-Bis (2-arsenophenylazo) chromotropic acid)
BoviLab CK	· Creatine phosphate disodium salt
BoviLab IP	· Inosine · Purine nucleoside phosphorylase

BoviLab Mg	· Xylidyl blue
BoviLab NEFA	· Acyl-CoA synthetase · Acyl-CoA oxidase

[Intended use]

Quantitatively measure the concentration of the following components in bovine whole blood, serum and plasma.

- AST (aspartate aminotransferase)
- Ca (calcium)
- CK (creatin kinase)
- IP (inorganic phosphorus)
- Mg (magnesium)
- NEFA (non esterified fatty acid)

[Measurement principle]

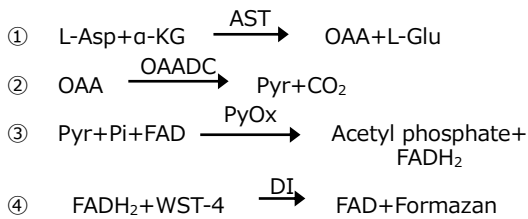
Whole blood dropped into the blood dripping area is pressurized by the device and passes through the blood cell separation membrane to separate the plasma. When plasma or serum is dropped directly, it passes through the blood cell separation membrane as it is. Plasma or serum moves to the reaction area and reacts by dissolving the applied and dried reagent. The coloration generated by the reaction is optically measured according to the measurement principle of each item. The measurement principle of each item in this product is as follows.

- **BoviLab AST**
It is measured by an enzymatic method using L-aspartic acid (L-Asp) and α-ketoglutaric acid (α-KG) as substrates.
 - ① AST present in the blood sample produces oxaloacetic acid (OAA) and L-glutamic acid (L-Glu) from L-Asp and α-KG
 - ② Oxaloacetate decarboxylase (OAADC) decomposes the resulting OAA to produce pyruvic acid (Pyr)
 - ③ Pyruvate oxidase (PyOx) produces FADH₂

from the resulting Pyr, the phosphoric acid (Pi) and flavin adenine dinucleotide (FAD)

④ The resulting FADH₂ acts on the reductive dye WST-4 in the presence of Diaphorase (DI) to produce the green formazan

The AST concentration in the blood sample is determined by measuring the coloration of the resulting formazan at a wavelength of 630 nm.

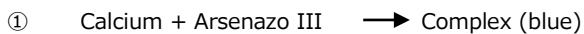


● BoviLab Ca

It is measured by the Arsenazo III method.

① Calcium in the blood sample binds to Arsenazo III to form a blue complex

The calcium concentration in the blood sample is determined by measuring the coloration of the resulting complex at a wavelength of 630 nm.



● BoviLab CK

It is measured by an enzymatic method using creatine phosphate (CP) and Adenosine-5'-diphosphate (ADP) as substrates.

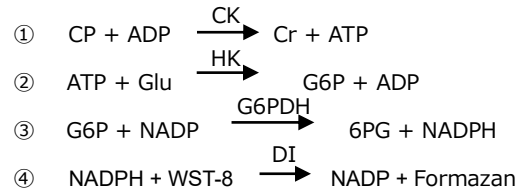
① CK present in the blood sample produces creatine (Cr) and adenosine-5'-triphosphate (ATP) from CP and ADP

② Hexokinase (HK) produces glucose-6-phosphate (G6P) and adenosine-5'-diphosphate (ADP) from the resulting ATP and glucose (GLU)

③ Glucose-6-phosphate dehydrogenase (G6PDH) produces the 6-phosphogluconic acid (6PG) and reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) from the resulting G6P and oxidized form of nicotinamide adenine dinucleotide phosphate (NADP)

④ The resulting NADPH acts on the reductive dye WST-8 in the presence of Diaphorase (DI) to produce the orange formazan

The CK concentration in the blood sample is determined by measuring the coloration of the resulting formazan at a wavelength of 560 nm.



● BoviLab IP

It is measured using an enzymatic method with purine nucleoside phosphorylase (PNP).

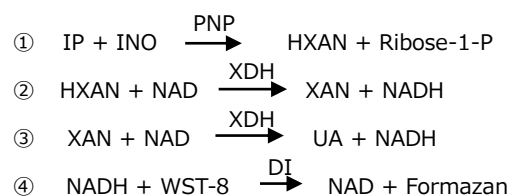
① IP in the blood sample reacts with inosine (INO) and breaks into hypoxanthine (HXAN) and ribose-1-phosphate (Ribose-1-P) using purine nucleoside phosphorylase (PNP)

② The resulting HXAN reacts with oxidized form of nicotinamide adenine dinucleotide (NAD) to produce xanthine (XAN) using xanthine dehydrogenase (XDH)

③ The resulting XAN becomes uric acid (UA), and at the same time, NAD changes to reduced form of nicotinamide adenine dinucleotide (NADH)

④ The resulting NADH acts on the reductive dye WST-8 in the presence of Diaphorase (DI) to produce orange formazan

The IP concentration in the blood sample is determined by measuring the coloration of the resulting formazan at a wavelength of 530 nm.



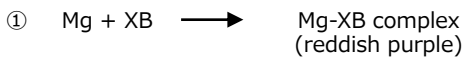
● BoviLab Mg

It is measured by the colorimetric method.

① Mg in the blood sample combines with

xylidyl blue (XB) under alkaline conditions to form a reddish purple complex

The Mg in the blood sample is measured by measuring the absorbance of the resulting complex at two wavelengths, 530 nm and 630 nm.



● Non-esterified fatty acid (NEFA)

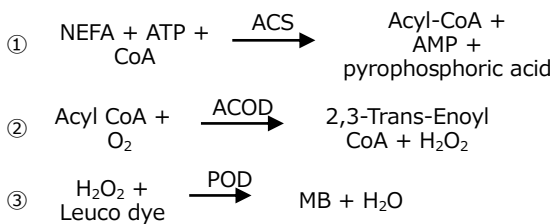
It is measured by an enzymatic method using Acyl-CoA synthetase (ACS), Acyl-CoA oxidase (ACOD) and Peroxidase (POD).

① NEFA in the blood sample reacts with adenosine-5'-triphosphate (ATP) and coenzyme A (CoA) to produce Acyl-CoA using ACS

② ACOD produces 2,3-Trans-enoyl CoA and hydrogen peroxide (H₂O₂) from the resulting Acyl-CoA and oxygen (O₂)

③ The resulting H₂O₂ acts on the phenothiazine-based leuco dye in the presence of POD to produce the blue-colored methylene blue (MB)

The NEFA concentration in the blood sample is measured by measuring the coloration of the resulting MB at a wavelength of 630 nm.



[Operational precaution]

1. Blood samples
 - (1) Measure immediately after blood collection.
 - (2) Heparin can be used as an anticoagulant. Do not use EDTA, citric acid, oxalic acid and sodium fluoride.
 - (3) If the measured value exceeds the upper limit of the measurement range, dilute it twice with saline solution and measure again. When diluted and measured, an error may occur, so treat it as estimation.
 - (4) When using a blood sample that has been refrigerated or frozen, bring it to room

temperature, invert carefully and mix it thoroughly before measuring.

2. Substances, drugs, etc. that affect the measurement results

● BoviLab Ca

- Be careful of calcium contamination.
- Using the gloves with calcium carbonate powder adhered to them may contaminate blood samples, resulting in positive error. Do not use gloves with calcium carbonate powder adhered.

● BoviLab CK

- CK is a very unstable enzyme, and its activity decreases due to freezing and thawing. Blood samples should be stored at 4 °C(39.2°F) and measured within 24 hours. If serum or plasma samples are stored for a long period of time, they should be measured within 48 hours even if stored frozen.

● BoviLab IP

- Avoid hemolysis when collecting the blood. Organic phosphorus in red blood cells breakdown easily to produce inorganic phosphorus. To avoid hemolysis after collecting the blood, measure immediately after collecting the blood. When measuring serum and plasma, complete the centrifugation within at least an hour after collecting the blood.
- Since the detergent may contain phosphorus, be careful not to leave any detergent on the equipment while cleaning.

● BoviLab NEFA

- Since lipoprotein lipase, phospholipase, and other enzymes that hydrolyze the lipids to produce fatty acids are present in the blood, measure immediately after collecting the blood.
- Preservation by freezing is preferable when storing the serum or plasma.

[Operation method]

1. Reagent preparation and handling

Remove this product from the refrigerator, bring it to room temperature, and then open the aluminum pouch. Once taken out of the

aluminum pouch, use the product as it is. In addition, open the aluminum pouch just before using the product.

2. Necessary apparatus/tools and materials/blood samples etc

BoviLab Blood analyzer

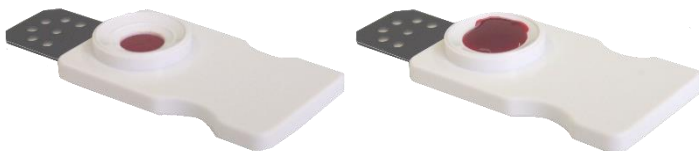
3. Measurement (operation) method

Drop approximately 150 µL of whole blood or plasma/serum onto the blood dripping area of the product, and immediately set it in the BoviLab Blood analyzer. The device reads the QR code affixed to the back of the product, automatically measures the blood sample, and displays the result. (For details on how to operate the BoviLab Blood analyzer, see the Quick Start Guide as well as the User Guide of the device.)

Appropriate blood sample dropping volume



Inappropriate blood sample dropping amount



Insufficient blood sample volume

Excessive blood sample volume

[Reference values]

Refer to the following website for reference range of each item in bovine blood.

<http://ib-holdings.com/en/bovilab-support>

[Clinical significance]

The metabolic profile test using blood test is used as a method to judge the health and nutritional status of the cattle. ^{1), 2)} Each of the specialized reagents in the BoviLab Blood analyzer is a reagent for analyzing blood components in dairy cattle and beef cattle and can be used for metabolic profile testing. Six tests can be performed simultaneously with one

reagent by using whole blood, plasma or serum. The BoviLab Blood analyzer can be used at farms and veterinary clinics to obtain analysis results.

[Performance]

Repeatability

The reproducibility range, when low-concentration and high-concentration controlled blood samples were measured 16 times simultaneously for 3 lots of each item, is as follows.

Items	Blood sample Concentration	C.V.(%)
AST	46 U/L	2.4-4.1%
	248 U/L	1.7-2.9%
Ca	5.58 mg/dL	1.5-3.8%
	12.68 mg/dL	1.5-2.7%
CK	338 U/L	3.5-6.4%
	719 U/L	2.7-4.9%
IP	2.32 mg/dL	1.4-2.3%
	8.50 mg/dL	1.0-2.3%
Mg	1.12 mg/dL	3.8-7.3%
	4.01 mg/dL	2.2-5.2%
NEFA	431 µEq/L	2.8-7.5%
	854 µEq/L	3.3-5.5%

Correlation

The correlation when measuring bovine plasma blood samples is as follows. Comparative method (X) is an automated analyzer.

Items	r	Regression equation	Concentration range
AST	0.995	$y=0.969x+2.94$	29-348 U/L
Ca	0.966	$y=1.001x-0.02$	4.64-12.13 mg/dL
CK	0.999	$y=0.992x+1.52$	79-6454 U/L
IP	0.991	$y=1.000x-0.02$	2.38-8.50 mg/dL
Mg	0.967	$y=0.937x+0.12$	1.11-4.72 mg/dL
NEFA	0.990	$y=0.971x-2.92$	79-1181 µEq/L

Measurement range

The measurement range of each item is as follows.

Items	Measurement range
AST	10-1000 U/L
Ca	1.50-16.00 mg/dL
CK	50-9999 U/L

IP	0.60-15.00 mg/dL
Mg	0.50-6.00 mg/dL
NEFA	50-1250 µEq/L

Interference of coexisting substances

No significant effects were observed up to the following concentrations for each of the substances listed below.

Items	Concentration	Hemoglobin (Hemolysis)	Bilirubin (Icterus)
AST	78 U/L	100 mg/dL	4 mg/dL
	300 U/L	500 mg/dL	4 mg/dL
Ca	5.86 mg/dL	500 mg/dL	4 mg/dL
	9.45 mg/dL	500 mg/dL	4 mg/dL
CK	97 U/L	significant interference	1 mg/dL
	471 U/L	significant interference	4 mg/dL
IP	5.42 mg/dL	250 mg/dL	4 mg/dL
	8.87 mg/dL	250 mg/dL	4 mg/dL
Mg	2.61 mg/dL	500 mg/dL	4 mg/dL
	4.35 mg/dL	500 mg/dL	4 mg/dL
NEFA	103 µEq/L	100 mg/dL	4 mg/dL
	757 µEq/L	200 mg/dL	4 mg/dL

There were no significant effects on Chyle (Turbidity) and ascorbic acid up to the following concentrations.

Chyle (Turbidity): 1000 formazin turbidity unit

Ascorbic acid: 2.0 mg/dL

[Information related to reference material on calibration]

The reference material applies to our company's basic rules, and cannot be used directly on this product.

Items	Reference material on calibration
AST	JCCLS certificated reference material Reference Standard-JSCC Enzyme
Ca	NIST SRM 915
CK	JCCLS certificated reference material Reference Standard-JSCC Enzyme
IP	NIST SRM 200
Mg	Specified reference material (National Metrology) compliant product
NEFA	In-house standard product

JCCLS: Japanese Committee for Clinical Laboratory Standards

NIST: National Institute of Standards and Technology

ReCCS : Reference Material Institute for Clinical Chemistry Standards

[Precautions for use and handling]

- Precautions for blood sample handling (hazard prevention)
 - Bovine blood samples are susceptible to Crimean-Congo hemorrhagic fever, Rift Valley fever, brucellosis, anthrax, etc. In order to avoid the risk of infection, wear disposable gloves when handling blood samples. Also, do not pipette by mouth.
 - Just like the blood sample, considering the risk of infection, handle the equipment carefully that comes in contact with the blood sample.
 - If the blood sample is scattered or adhered to any surface, wipe it off and disinfect the surface thoroughly with a disinfectant such as Hypochlorous acid (effective chlorine concentration 1,000 ppm, 0.1%) or 80% ethanol.
- Precautions to be taken when using slides
 - BoviLab Slides that have been brought back to room temperature should be stored at room temperature and used within 3 days. They cannot be returned to refrigeration and stored again.
 - Do not freeze this product. Do not use if stored in the freezer, as it may not give correct results.
 - Do not use this product after the expiration date.
 - Use a new product for each measurement. Do not reuse the used reagent slide.
 - If the aluminum pouch is damaged, do not use it. Moreover, when opening the outer box, take care not to damage the aluminum pouch present inside.
 - Aluminum pouch contains this product and desiccant. Do not use this product if it does not contain a desiccant.
 - Do not directly touch the blood sample supply section (blood dripping area) and the reaction section of the product.

(8) Do not stain the label (QR code) on the back of this product.

(9) Use following method to disinfect used products and equipments that came into contact with blood samples.

- Disinfect with Hypochlorous acid (effective chlorine concentration 1,000 ppm or more, and soaked for 1 hour or more)
- Disinfection treatment using glutaraldehyde (2%, soaked for an hour or more)
- Autoclave sterilization (121 °C(249.8°F), for 20 minutes or more)

(10) Do not reuse this product or its packaging for any other purpose.

3. Precautions when disposing the slides

(1) When disposing of this product, consider the risk of infection and dispose of the product by incineration, melting, sterilization, disinfection, etc. according to local rules and regulations and at the responsibility of each facility.

[Storage method/validity period]

1. Storage method

Store in a refrigerator at (2-8°C(35.6-46.4°F)).

2. Validity period

6 months to 1 year from the date of manufacture (depending on the item configuration)

The expiration date is stated on the outer box as well as on the individual packaging.

[Product Specifications]

Size of

the aluminum pouch: W 60 mm × L 90 mm

the slide: W 20 mm × L 50 mm

Weight: Slide main unit: 2.3 g

Material: ABS resin/polyethylene terephthalate (PET)/glass fiber/cellulose acetate film/polycarbonate film

Made in Japan

[Package unit]

15 slides/box

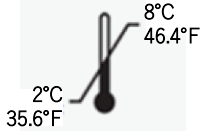
[References]

1) Keiji Okada. *Juunaikagaku the 2nd edition Daidoubutuhēn* : Chapter17, Seisann juuiryo sisutemu(生産獣医療システム/PRODUCTION MEDICINE SYSTEM), 2014, Bun-Eido Publishing Co.,Ltd., p.359-369

ISBN:978-4-8300-3252-3

2) Fukumori R, Taguchi T, Oetzel GR, Oikawa S. *Res Vet Sci.* 2021;135:247-252

[Explanation of symbols]

Symbols	Title
	Store refrigerated (2-8°C) (35.6-46.4°F)

[Contact information/Manufacturer]



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