S100A8/S100A9 heterodimer (human)

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S100A8/S100A9 heterodimer (human)

ELISA kit

#A05083.96 wells

For research laboratory use only
Not for human diagnostic use

This assay has been developed
& validated by Bertin Bioreagent

Fabriqué en France
Made in France

#A11083

Version: 0121

Table of contents

96 wells

Storage: +4°C

Expiry date: stated on the package

This kit contains:

Designation	Colour of cap	Item #	Quantity per kit	Form
S100A8/S100A9 heterodimer (human) precoated 96-well Strip Plate	Blister with zip	A08083.1 ea	1	-
Streptavidin Poly_HRP Tracer	Green	A04410.100 dtn	1	Liquid
S100A8/S100A9 heterodimer (human) Biotin-labelled Antibody	Red	A03083.100 dtn	1	Lyophilised
S100A8/S100A9 heterodimer (human)Standard	Blue with red septum	A06083.1 ea	2	Lyophilised
S100A8/S100A9 heterodimer (human) Quality Control	Green with red septum	A010083.1ea	2	Lyophilised
Biotin-free ELISA Buffer	Grey / Blue	A07410.1 ea	1	Lyophilised
Wash Buffer	Silver	A17000.1 ea	1	Liquid
Tween 20	Transparent	A12000.1 ea	1	Liquid
HRP Substrate Solution	Black	A09034.100 dtn	1	Liquid
HRP Stop Solution	Yellow	A22410.100 dtn	1	Liquid
Technical Booklet	-	A11083.1 ea	1	-
Well 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 36 samples in duplicate. If you want to use the kit in two times, we provide one additional vial of Standard and one of Quality Control.

Precaution for use

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

Each time a new pipette tip is used, aspirate a sample or reagent and expel 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 human 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 solutions. 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 materials and samples.

Temperature

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

Background

S100A8 and S100A9 are Ca^{2+} binding proteins that belong to the S100 family, called calgranulin. Calgranulins are endogenous molecules released in response to environmental triggers and cellular damage. Also known as Damage-Associated Molecular Pattern Molecules (DAMPs), these proteins play an important role in a diverse range of physiological and pathological processes, such as host defense, wound healing, autoimmunity, oncogenesis and inflammation, among others (Kerkhoff et al 2014; Holzinger et al 2018).

S100A8 (S100 calcium-binding protein A8, calgranulin A, MRP8) and S100A9 (S100 calcium-binding protein A9, calgranulin B, MRP14) subunits are unique within the calgranulin family because they preferentially form a heterodimer. This heterodimer is termed calprotectin, based on its role in innate immunity. Human S100A8 and S100A9 are mainly derived from immunocytes, such as neutrophils, macrophages and monocytes (Crowe et al 2019; Catalán et al 2018). They consist of 93 and 113 amino acid residues, respectively, and S100A9 has a truncated isoform with 110 amino acids. Extracellular S100A8 and S100A9 bind pattern recognition receptors (PRRs) including Toll-like receptors

(TLRs) and receptor for advanced glycation end products (RAGE) to activate the innate immune system and mediate inflammation by influencing monocyte and macrophage behavior (Wang et al 2018).

Given that S100A8 and S100A9 are intensely upregulated during trauma, infection, heat, stress and many other inflammatory processes, their heterodimer is a valuable candidate as both a diagnostic biomarker and a therapeutic target for inflammation-associated diseases (Dubois et al 2020). Plasma levels of S100A8/S100A9 and S100A12 were found to be higher in septic shock patients than in healthy volunteers. Furthermore, the high level of plasma calgranulins at admission in septic shock, were higher in non-survivors compared to survivors (Dubois et al 2019).

Principle of the assay

This Enzyme Immunometric Assay (EIA/ELISA) is based on a sandwich technique. The wells of the plate are coated with a monoclonal antibody specific to S100A8/S100A9 heterodimer (human).

S100A8/S100A9 heterodimer (human) introduced into the wells (standard or sample) will be bound by the monoclonal polyclonal antibody coated on the plate and is then detected by a second monoclonal antibody tagged with biotin also specific for S100A8/S100A9 heterodimer (human).

The two antibodies then form a sandwich by binding on different parts of the S100A8/S100A9 heterodimer (human).

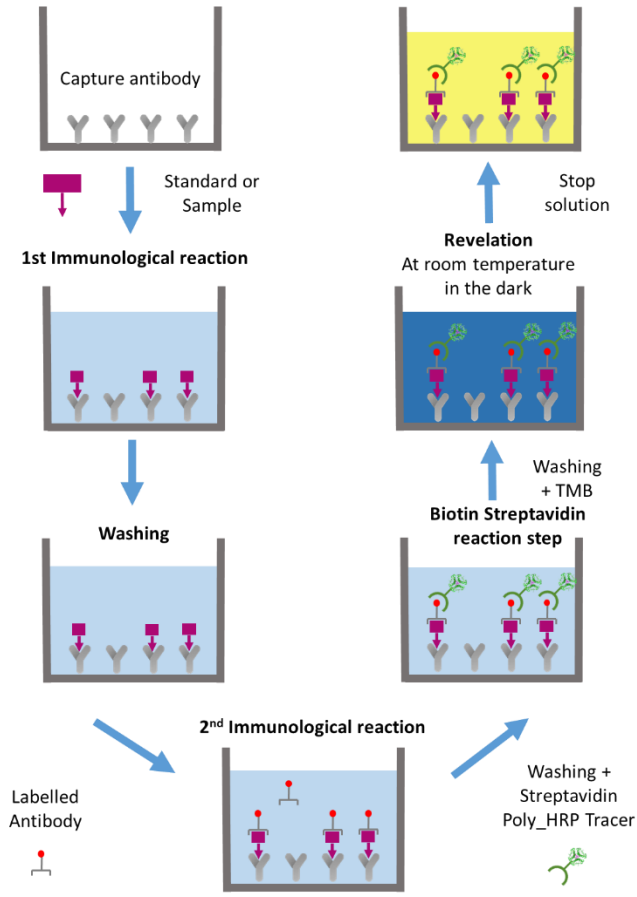
The sandwich is immobilised on the plate so reagents in excess may be washed away.

The immunological complex is revealed by the interaction between biotin and streptavidin labelled with HRP (Tracer).

The concentration of S100A8/S100A9 heterodimer (human) 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 S100A8/S100A9 heterodimer (human) present in the well during the immunological incubation.

The principle of the assay is summarised below:



Materials and equipment required

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

For the assay:

- Precision micropipettes (20 to 1000 µL)
- Spectrophotometer plate reader (450 nm filter)
- Microplate washer (or washbottles)
- Orbital microplate shaker
- Multichannel pipette and disposable tips 30-300µL
- UltraPure water ELISA Grade #A07001
- Polypropylene tubes

Water used to prepare all ELISA reagents and buffers must be UltraPure (deionized & free from organic contaminant traces).
Do not use distilled water, HPLC-grade water or sterile water.

- UltraPure water ELISA Grade may be purchased from Bertin Bioreagent (item #A07001).

The shaking speed is an important parameter and it must vigorous enough to agitate well content and without splashing droplets into the other wells (around 500 rpm, speed to be adapter do the device)

Sample type, collection and preparation

This assay has been validated to measure S100A8/S100A9 heterodimer (human) in human serum.

It is the responsibility of the user to check the compatibility of the assay with the analysed matrix.

General precautions

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

Sample collection

Blood samples are collected in dry tubes.

Sample preparation

Serum sample must be diluted at least at 1:100 in 1x Biotin-free ELISA Buffer before the assay. The dilution of highly concentrated samples should be optimized by the user.

Reagent preparation

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

the assay of 36 samples in duplicate.

If you want to use the kit in two times, we provide one additional vial of Standard and one of Quality Control.

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

1x Biotin-free ELISA Buffer

Reconstitute the Biotin-free ELISA Buffer #A07083 with 50 mL of UltraPure water ELISA Grade. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.
Stability at 4°C: 3 weeks.

S100A8/S100A9 heterodimer (human) Standard

Reconstitute one S100A8/S100A9 heterodimer (human) Standard vial #A06083 with 1 mL of 1x Biotin-free ELISA Buffer. Allow it to stand for 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

The concentration of the first standard (S1) is 80.0 ng/mL. Prepare seven polypropylene tubes (for the seven other standards) and add 500 µL of 1x Biotin-free ELISA Buffer into each tube. Then prepare the standards by serial dilutions as follow:

Standard	Volume of Standard	Volume of 1x Biotin-free ELISA Buffer	Standard concentration
S1	-	-	80.0 ng/mL
S2	500 µL of S1	500 µL	40.0 ng/mL
S3	500 µL of S2	500 µL	20.0 ng/mL
S4	500 µL of S3	500 µL	10.0 ng/mL
S5	500 µL of S4	500 µL	5.0 ng/mL
S6	500 µL of S5	500 µL	2.5 ng/mL
S7	500 µL of S6	500 µL	1.3 ng/mL
S8	500 µL of S7	500 µL	0.6 ng/mL

Stability at 4°C: With in the day.

S100A8/S100A9 heterodimer (human) Quality Control

Reconstitute one S100A8/S100A9 heterodimer (human) QC vial #A10083 with 1 mL of 1x Biotin-free ELISA Buffer. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.
Stability at 4°C: With in the day

S100A8/S100A9 heterodimer (human) Biotin-labelled Antibody

Reconstitute the S100A8/S100A9 heterodimer (human) Biotin-labelled Antibody vial #A03083 with 10 mL of 1x Biotin-free ELISA Buffer. Allow it to stand 5 minutes until

completely dissolved and then mix thoroughly by gentle inversion.
Stability at +4°C: 3 weeks.

Wash Buffer

Dilute 2 mL of concentrated Wash Buffer #A17000 with 800 mL of UltraPure water ELISA Grade. Add 400 µL of Tween 20 #A12000. Use a magnetic stirring bar to mix the content.
Stability at +4°C: 3 weeks.

Assay procedure

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

Plate preparation

Prepare the Wash Buffer as indicated in the reagent preparation section.

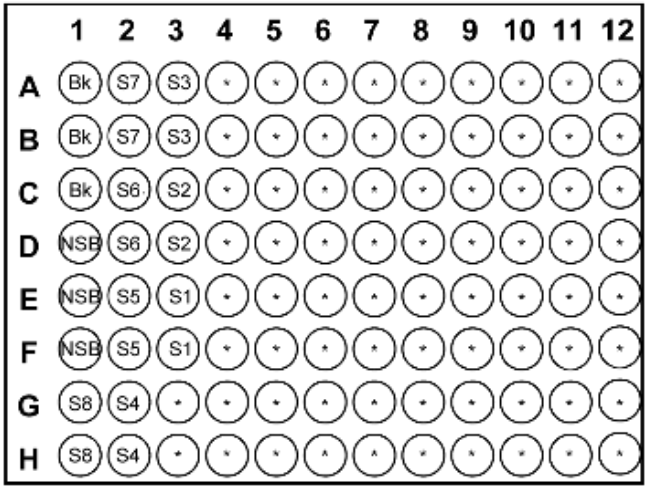
Open the plate pouch and select enough strips for your assay and place the unused strips back in the pouch.
Stability at +4°C: 3 weeks.

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 hereafter. The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.



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

Pipetting the reagents

All samples and reagents must reach room temperature prior to performing the assay.
Use different tips to pipet the buffers, standards, samples, antibody and other reagents.

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

> ***1x Biotin-free ELISA Buffer***

Dispense 100 µL to Non Specific Binding wells (NSB) wells.

> ***S100A8/S100A9 heterodimer (human) Standard***

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

> ***S100A8/S100A9 heterodimer (human) QC and Samples***

Dispense 100 µL in duplicate to appropriate wells. See section "Sample type, collection and preparation" for dilution of samples.

Incubating the plate

Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking on an orbital microplate shaker (See shaking speed advices page 11).

Washing the plate

Rinse each well 5 times with 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

> ***S100A8/S100A9 heterodimer (human) 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 on an orbital microplate shaker (See shaking speed advices page 11).

Washing the plate

Rinse each well 5 times with 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

> ***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 on an orbital

microplate shaker (See shaking speed advices page 11).

Developing and reading the plate

- Empty the plate by turning it over. 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 100µL of HRP Substrate Solution to each well.

- Incubate the plate in the dark at room temperature **without shaking**. ***To set up the incubation time, please refer to the Quality Control Sheet (QCS) corresponding to the lot#***.

- Add 100µL 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).

Assay procedure summary

Enzyme Immunoassay Protocol (volumes are in µL)				
	Blank	NSB	Standard	Sample or QC
1x Biotin-free ELISA Buffer	-	100	-	-
Standard	-	-	100	-
Sample or QC	-	-	-	100
Cover plate, incubate **60** minutes at room temperature under orbital shaking*				
Wash strips 5 times with 300 µL/well Discard liquid from the wells & dry on absorbent paper				
Biotin-labelled Antibody	-	100		
Cover plate, incubate **60** minutes at room temperature under orbital shaking*				
Wash strips 5 times with 300 µL/well Discard liquid from the wells & dry on absorbent paper				
Streptavidin Poly_HRP Tracer	-	100		
Cover plate, incubate **30** minutes at room temperature under orbital shaking*				
Wash strips 5 times with 300 µL/well Discard liquid from the wells & dry on absorbent paper				
HRP Substrate Solution	100			
Incubate the plate in the dark without agitation				
HRP Stop Solution	100			
Read the plate at 450 nm				

*The shaking speed is an important parameter and it must vigorous enough to agitate well content and without splashing droplets into the other wells (around 500 rpm, speed to be adapter do the device).

Data analysis

Make sure that your plate reader has subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate. If not, do it at this steps.

- Calculate the average absorbance for each NSB, standard, QC and sample.
- 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 80.00 ng/mL should be re-assayed after dilution in 1x Biotin-free ELISA Buffer.
- Most plate readers are supplied with a curve-fitting software capable of graphing these data (4-parameter logistic fit 4PL). If you have this type of software, we recommend using it. Refer to it for further information.

Two vials of Quality Control are provided with this kit.
Your standard curve is validated only if the calculated concentration of the Quality Control obtained with the assay is +/- 25% of the expected concentration (see the label of the QC vial)

Acceptable range

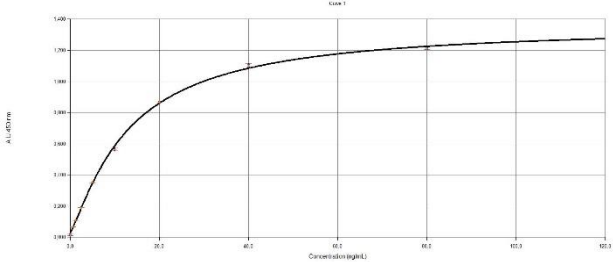
- NSB absorbance ≤ 0.200 A.U.
- Limit of detection ≤ 0.6 ng/mL
- QC ±25% of the expected concentration (see the label of the QC vial)
- Absorbance values (blank-deducted) of the standard S1 ≥ 1.1
- Ratio: [Absorbance values (blank-deducted) of the standard S8] / [Absorbance values (blank-deducted) of the NSB] ≥ 1.2
- Curve analysis method
  - recommended: 4 parameter logistic fitting (4PL)
  - alternative: 4 parameter logistic fitting with a 1/Y ponderation (4-PL 1/Y)

Typical results

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: 20 minutes developing at room temperature, reading at 450 nm. A 4 parameter logistic fitting was used to determine the concentrations.

Standard	S100A8/S100A9 heterodimer (human) ng/mL	Absorbance A.U.
S1	80.0	1.213
S2	40.00	1.102
S3	20.0	0.867
S4	10.0	0.565
S5	5.0	0.354
S6	2.5	0.191
S7	1.3	0.111
S8	0.0	0.068
NSB	0.00	0.017

Typical S100A8/S100A9 heterodimer (human) standard curve



Assay characteristics

- ***Validated for***
  - Human serum samples
- ***Limit of detection (LOD):*** ≤0.6 ng/mL (calculated as the concentration of *S100A8/S100A9 heterodimer (human)* corresponding to the NSB average plus three standard deviations)
- ***Cross-reactivity***

Molecule/Species	Cross-reactivity
S100A12 (human)	Not detected

- ***Inter-assay variation***

QC level	QC 1	QC 2	QC 3
Mean concentration (ng/mL)	6.0	14.7	55.4
Mean concentration (ng/mL) x dilution	603.0	1469.6	5542.8
CV %	6.4	3.9	10.0

QC are sera spiked or un-spiked with S100A8/S100A9 heterodimer (human) and then diluted at 1:100 (10 µl QC +

990 µL of 1x Biotin-free ELISA Buffer) before testing.

For the inter–assay validation, the number of replicates is equal to 6 for each level of QC. The three validation levels were analyzed along with the calibration curve for a total of 6 independent runs.

> ***Intra-assay variation***

QC level	QC 1	QC 2	QC 3
Mean concentration (ng/mL)	6.1	14.4	56.5
Mean concentration (ng/mL) x dilution	608.5	1444.0	5649.6
CV %	7.1	4.1	11.3

QC are sera spiked or un-spiked with S100A8/S100A9 heterodimer (human) and then diluted at 1:100 (10 µl QC + 990 µL of 1x Biotin-free ELISA Buffer) before testing.

For the intra–assay validation, the number of replicates is equal to 10 for each level of QC. The three validation level were analyzed along with the calibration curve for a unique experiments.

> *Matrix variability*

Matrix#	Mean endogenous conc. x dil. factor (1) (ng/mL)	Mean spiked matrix conc. x dil. factor (1) (ng/mL)	Back calculated conc. (ng/mL)	Back calculated spiked conc. (Relative Error)
1	741.7	3881.4	3139.7	6.7 %
2	1380.3	4881.1	3500.8	19.0 %
3	1997.4	4927.7	2930.3	-0.4 %
4	1641.8	4529.9	2888.1	-1.9 %
5	3208.2	5477.8	2269.6	-22.9 %
6	119.2	3894.7	2703.5	- 8.1 %
7	3023.6	5787.0	2763.4	-6.1 %
8	898.2	4105.6	3207.4	- 9.0 %
9	1032.6	4009.8	2977.2	1.2 %

(1) Matrix dilution is 1:100

9 individual sera samples were spiked and not with S100A8/S100A9 heterodimer (human). Each sample (spiked or not) was evaluated 100 fold diluted in duplicate and analyzed against a calibration curve.

> *Linearity*

Three individual sera were spiked or not with S100A8/S100A9 heterodimer (human) and diluted at 1:100 Each spiked diluted sample is evaluated on 5 serial dilutions

Matrix#	Endogenous Conc. (ng/mL)	Dilution	Spiked S100A8/S100A9 heterodimer (human) (ng/mL)	Mean of spiked conc. x dil. (ng/mL)	CV % Mean of spiked conc x dil	Recovery %	Mean Recovery %
1	5.1	1	80.2	80.2	10.6	125.6	103.6
		2	32.1	64.2		100.6	
		4	16.2	64.9		101.6	
		8	7.6	60.9		95.4	
		16	3.9	62.9		98.4	
		26	2.5	63.7		99.8	
2	6.8	1	75.8	75.8	10.0	115.7	99.3
		2	35.0	70.0		106.8	
		4	15.6	62.6		95.5	
		8	7.4	58.8		89.8	
		16	3.9	62.1		94.8	
		26	2.3	60.9		92.9	

Matrix	Endogenous Conc. (ng/mL)	Dilution	Spiked S100A8/S100A9 heterodimer (human) (ng/mL)	Mean of spiked conc. x dil. (ng/mL)	CV % Mean of spiked conc. x dil.	Recovery %	Mean Recovery %
3	34.5	1	92.8	92.8	4.9	99.6	92.6
		2	43.7	87.3		93.7	
		4	20.5	82.2		88.1	
		8	10.2	81.3		87.2	
		16	5.4	86.2		92.5	
		26	3.4	87.9		94.3	

> *Parallellism*

Five individual human sera samples were diluted between 1:100 and 1:1600 by serial dilution. Each dilution was analysed against a calibration curve.

Matrix#	Dilution factor	S100A8/S100A9 heterodimer (human) conc. (ng/mL)	S100A8/S100A9 heterodimer (human) conc. (ng/mL) X Dilution factor	CV%
1	100	14.1	1412.9	7.6 %
	200	7.1	1427.8	
	400	3.8	1538.4	
	800	2.0	1614.4	
	1600	1.1	1683.2	
2	100	39.0	3900.4	5.0 %
	200	17.1	3420.8	
	400	8.9	3554.8	
	800	4.5	3612.0	
	1600	2.3	3718.4	
3	100	8.5	854.6	7.6 %
	200	4.2	841.4	
	400	2.1	857.6	
	800	1.1	863.2	
	1600	0.4	715.2	
4	100	27.4	2737.7	1.3 %
	200	13.5	2699.4	
	400	6.8	2730.8	
	800	3.5	2771.2	
	1600	1.7	2887.2	
5	100	9.6	956.3	2.0 %
	200	4.9	974.4	
	400	2.5	988.0	
	800	1.2	968.8	
	1600	0.6	937.3	

Troubleshooting

Absorbance values are too low:

- one reagent has not been dispensed,
- too low shaking,
- 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.

These are a few examples of troubleshooting that may occur. If you need further explanation, Bertin Bioreagent will be happy to assist you. Feel free to contact our Technical Support staff by phone +33 (0)139 306 036 or E-mail tech@bertin-bioreagent.com, and be sure to indicate the batch number of the kit (see outside the box).

Bertin Bioreagent proposes ELISA Training kit #B05005. For further information, please contact our Technical Support.

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With 30 years of experience, Bertin Bioreagent develops and sells best-in-class kits and products for life science research labs. Our scientist team innovate each day to tailor biomarker assays, pre-analytical products, kits, antibodies and biochemicals that are ready to use, fully validated with a strict quality control.
We strive to address a broad range of research interest: inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, pain, prion diseases.

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Should you need help with a product, you can contact our technical support by emailing to tech@bertin-bioreagent.com
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