EN

User Manual

MONOSCREEN Quant ELISA

BIO K 420 NO (EN) V1.2
15-07-2020

MonoScreen QuantELISA Immunoglobulin Easy

Reference : BIO K 420

Competitive ELISA test for the quantification of immunoglobulins

For veterinary *in vitro* use only

Sample	Bovine*	Equine*	Swine
Blood serum and plasma	✓	✓	-
Colostrum	✓	-	✓

* animal < 15 days

To place an order

Product Reference	BIO K 420/1
Format	1 plate, 12X8-well strips
Reagents	96

Composition of kit

	BIO K 420/1
Microplates	1
Washing Solution (20X)	1 X 100ml
Colored dilution buffer (1X)	1 X 30ml
Conjugate (50X)	1 X 0,3ml
Standard	1 X 0,35ml
Single Component TMB Solution (1X)	1 X 12ml
Stop Solution (1X)	1 X 6ml

Revision history

23/06/2020 - V1.1	text formatting
15/07/2020 - V1.2	Adding equations (chapter I) and Notes formating

Note : minor changes concerning the typography, grammar and format are not included in the revision history.

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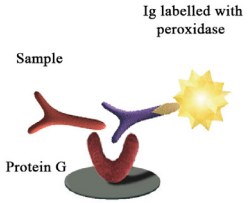
A. Introduction

Colostral immunity is an important factor of survival in young animals. It is based on 3 conditions: the quality of the colostrum (concentration of immunoglobulins), the quantity transferred and the early timing of ingestion after birth. The ELISA kit from Bio-X Diagnostics is intended for the measurement of immunoglobulins in colostrum (bovine and porcine) or in blood serum (bovine and equine).

B. Test principle

The microplates were sensitized with protein G specific for immunoglobulins. The samples and standard are added to the wells of the microplate at the same time as the conjugate. After incubation and washing of the preparation, the substrate solution (single component TMB) is added. The staining intensity is inversely proportional to the immunoglobulin concentration in the sample. Reading is performed at 450 nm.



C. Additional materials and equipment required but not provided

- Distilled/demineralised water
- Single or multi-channel pipette with accuracy (range 2-20µl, 20-200µl and 100-1000µl) and disposable tips
- Microplate Reader (filter 450nm)
- Microplate washer and shaker (optional)
- Dilution Microplate
- Standard laboratory material : graduated cylinder, tube holder, lid,...

D. Precautions for use

- Store reagents between +2 and + 8 ° C. The washing solution can be stored at room temperature.
- Keep unused strips in the sealed aluminum pouch with its desiccant.
- Do not use reagents beyond the expiration date.
- Do not use reagents from other kits.
- Monitor the quality of the water used.
- The stop solution contains 1 M phosphoric acid. Handle this product with caution.
- Dispose of the equipment used in accordance with current legislation on environmental protection and management of biological waste.
- Keep the TMB solution away from light

E. Solution Preparation

- The washing solution must be diluted 20 times in distilled / demineralized water. The solution crystallizes spontaneously when cold. Bring the vial to 21 ° C +/- 3 ° C for the crystals to disappear; mix the solution carefully and collect the necessary volume.
- The dilution buffer is ready to use. The dilution buffer is yellow.
- The conjugate is to be diluted 50 times in the dilution buffer.
- The stop solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless. If a blue color is visible, this would indicate a contamination of the solution or the pipette

F. Sample Preparation

Prepare dilutions of samples and standard in PBS buffer using the table below.
The accuracy of the measurement depends largely on the dilution steps.

Matrix	Final Dilution	Recommended dilution			
		Individual Dilution	Number of tubes	Volume to transfer	Volume of PBS per tube
Standard	500	22,4	2	25 µl	535 µl
Calf serum <15 jours	100	10	2	50 µl	450 µl
Bovine Colostrum	1000	31,6	2	25 µl	765 µl
Adult bovine serum	500	22,4	2	25 µl	535 µl
Swine Colostrum	1000	31,6	2	25 µl	765 µl
Foal serum <15 jours	400	20	2	25 µl	475 µl

G. Procedure

- All components must be brought to 21 ° C +/- 3 ° C before use.
- Read previous points carefully.

1. In a dilution microplate, distribute the **diluted standard twice** with 100 µl per well. **Dispense 100 µl** of the dilutions of samples to each well.
2. Add **100 µl of diluted conjugate** to each well in the dilution microplate. Avoid touching samples in the wells with the microtips while adding the conjugate
3. Shake carefully the dilution microplate.
4. Transfer 100 µl from the dilution microplate to the microplate of the kit using a multichannel pipette. Be sure to change the tips between two rows of samples.
5. Cover and incubate the plate at **21 ± 3°C** for **60 ± 5 min**.
6. Dispose of the contents of the microplate. **Wash the microplate 3 times with 300 µl of washing solution.** Avoid the formation of bubbles in the wells and the drying of the microplate between each wash
7. Dispense **100 µl of TMB solution** into each well.
8. Incubate **10 ± 1 min at 21 ± 3°C** away from light, without covering.
9. Dispense **50 µl of stop solution into each well**. The color changes from blue to yellow.
10. Record optical densities with a plate spectrophotometer using a filter 450 nm within 5 minutes of adding the stop solution.

H. Result Validation

The test can only be validated if :

- The mean optical density (OD) value of the standard is greater than 0.800 and lower than 1.600.
- The difference in optical density (OD) between the two standards is less than 0.250.

I. Result Interpretation

Get a quick and easy interpretation of your results with our free online platform **AnalysiScreen** available on our website at : https://www.biox.com

AnalysiScreen will calculate your results using the kit standards and the pre-calculated calibration curves internal to the platform.

To calculate the immunoglobulin concentrations of samples without AnalysiScreen:

1. Calculate its coefficient for each sample by applying the following formula :

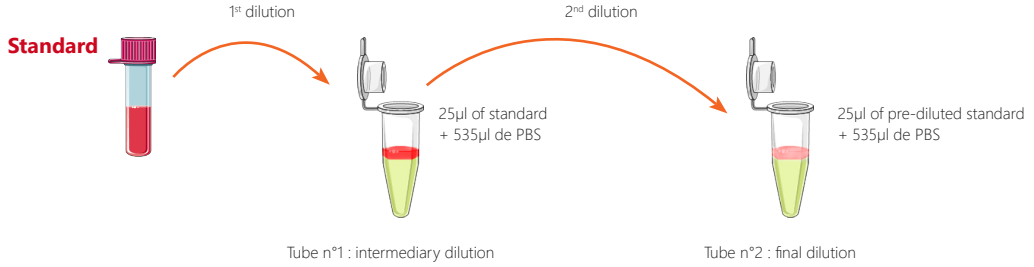
$$Y = Coeff.\ sample. = \frac{OD_{sample}}{OD_{standard\ mean}}$$

2. Then calculate the concentration of each sample using the following formula :

$$Concentration\left(^{mg}/_{ml}\right) = c * \left(\frac{a-d}{Y-d} - 1\right)^{\frac{1}{b}} * \frac{dilution}{1.000.000}$$

You will find the parameters *a*, *b*, *c* and *d* in the certificate of analysis provided.

Example of recommended preparation



Notes*

Sample dilution
2X standard dilution 1/500

Add 100 µl of sample + 100 µl of conjugate in **dilution microplate**

Mix

Transfer 100 µl of mix from the **dilution microplate** to the **microplate of the kit**

Room Temp. | 60 min | 3X Wash

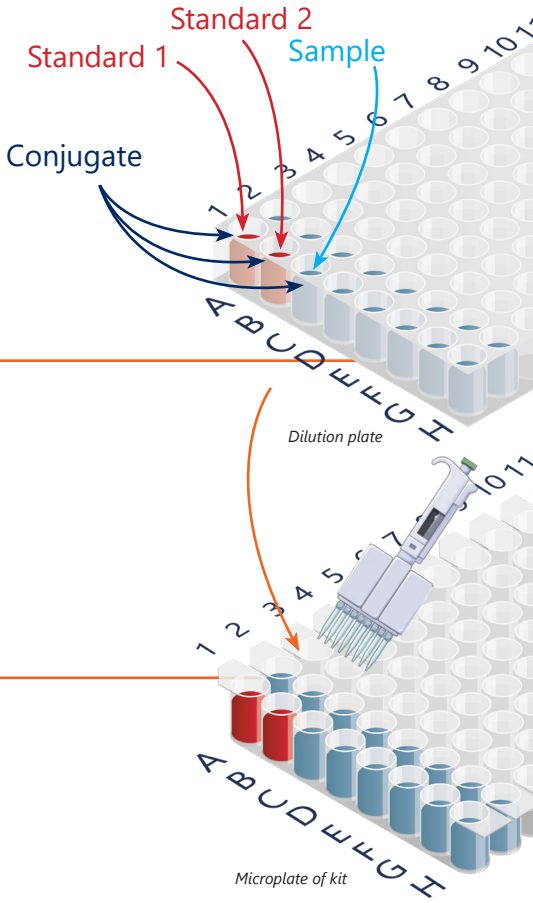
Add 100 µl of TMB

Room Temp. | 10 min | Obscurity

Add 50 µl of Stop solution

Record optical densities

450 nm



* Notes are a summary of the instructions for use and cannot substitute the latter