MONOSCREEN Quant ELISA

Instruction manual
**BIOK420-IgG bovines Easy_NO_(EN)_V02**
**10/10/2023**

# Monoscreen QuantELISA Immunoglobulin Easy

Reference: BIO K 420

ELISA test for the quantification of immunoglobulins

Monowell, competition test

For veterinary *in vitro* use only

| Sample | Bovine | Equine | Swine |
|---|---|---|---|
| Serum | 1-25 mg/mL | 3-30 mg/mL* | ✘ |
| Colostrum | 20-150 mg/mL | ✘ | 20-150 mg/mL |
| Milk | 0,1-1 mg/mL | ✘ | ✘ |

*animal < 15 days

## Presentation

| Product reference | BIO K 420/1 |
|---|---|
| Format | 1 plate, strips of 8 wells |
| Reactions | 96 tests |

## Kit composition

| Matériels fourni | BIO K 420/1 |
|---|---|
| Microplate | 1 |
| Washing solution (20X) | 1 x 100 mL |
| Colored dilution buffer (1X) | 2 x 100 mL |
| Single component TMB Solution (1X) | 1 x 12 mL |
| Stopping solution (1X) | 1 x 6 mL |
| Conjugate (50X) | 1 x 0,3 mL |
| Standard | 1 x 0,35 mL |

## Revision history

| Date | Version | Modifications |
|---|---|---|
| 23/06/2020 | V1.1 | Text formatting |
| 15/07/2020 | V1.2 | Adding equations (chapter I) and notes formatting |
| 25/01/2021 | V1.3 | Modifying the equation and adding a range of results |
| 11/02/2021 | V1.4 | Removal of the matrix "adult bovine serum", addition of the PBS buffer preparation |
| 08/03/2021 | V1.5 | Addition of milk matrix for bovine and modification of paragraph F-Sample preparation |
| 10/10/2023 | V02 | Layout and simplification of the entire manual |

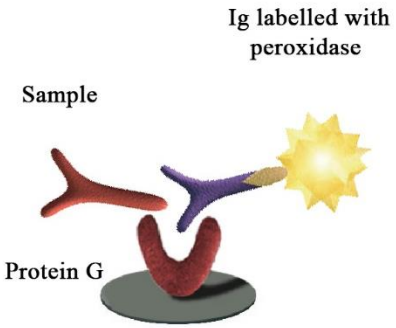
Note : minor typographical, grammar and formatting changes are not included in the revision history.

## 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. Material required but not provided

- Distilled/demineralized 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. Warnings and precautions of 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. Preparation of the solutions

- The washing solution must be diluted 20 times in distilled/demineralized water. The solution crystallizes spontaneously when cold. Bring the vial to 21±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. Preparation of the samples

Prepare the dilution of the samples and standard in the dilution buffer using the table at the bottom of the page.
The accuracy of the measurement depends largely on the dilution steps.

## G. Procedure

- Bring all the reagents to 21±3°C before use.
- Carefully read through the previous points.

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. Carefully shake the dilution microplate.
3. 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. Cover and incubate the plate at **21±3°C** for **60±5min**.
4. Remove the content of the microplate. **Wash the microplate 3 times** with **300 µL of washing solution** per well. Avoid the formation of bubbles in the wells between each wash.
5. Distribute **100 µL** of **TMB solution** per well. Incubate at **21±3°C** for **10±1min**, away from the light without covering.
6. Distribute the **stopping solution** at a rate of **50 µL** per well. The color changes from blue to yellow.
7. Record optical densities with a plate spectrophotometer using a **450nm** filter within **5 minutes** after adding the stopping solution.

| | | | Recommended dilution | | | |
|---|---|---|---|---|---|---|
| Matrix | Factor Z | Final dilution | Individual dilution | Number of tubes | Volume to transfer | Volume of dilution solution per tube |
| Standard | - | 500 | 22,4 | 2 | 25 µL | 535 µL |
| Calf serum <15 days | 1,120 | 100 | 10 | 2 | 50 µL | 450 µL |
| Bovine colostrum | 1,425 | 1000 | 31,6 | 2 | 25 µL | 765 µL |
| Swine colostrum | 1,000 | 1000 | 31,6 | 2 | 25 µL | 765 µL |
| Foal serum <15 days | 0,850 | 400 | 20 | 2 | 25 µL | 475 µL |
| Bovine milk | 1,000 | 10 | 10 | 1 | 25 µL | 225 µL |

## H. Validation of results

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. Interpretation of results

**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:

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

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

You can find the parameter *Z* and the dilution in the table on page 2.

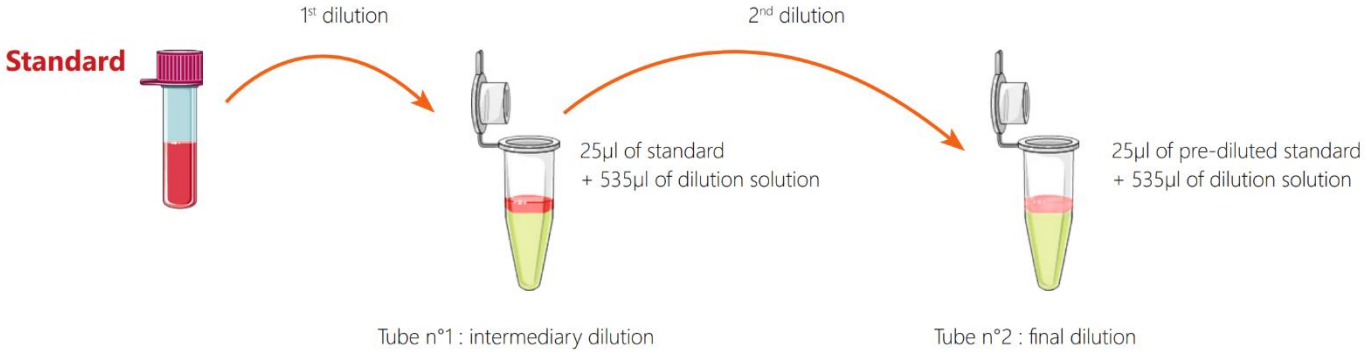
Get the interpretation of your results quickly and easily using **AnalysiScreen,** our free online platform, available on our website: https://www.biox.com.

AnalysiScreen™ is the new module for reading and interpreting all types of Monoscreen™ and Multiscreen™ ELISA plates. **Analysiscreen™** is :

- Free
- Accessible online via our website: https://www.biox.com
- Updated in real time
- Compatible with all Bio-X Diagnostics plate designs
- Very easy to use

SCAN ME

*Example of recommended preparation*

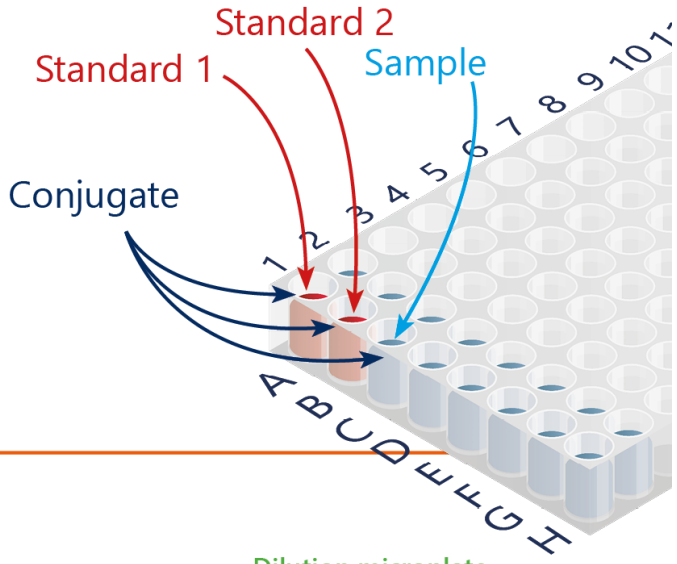


## Notes*

Sample dilution
2X standard dilution 1/500

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

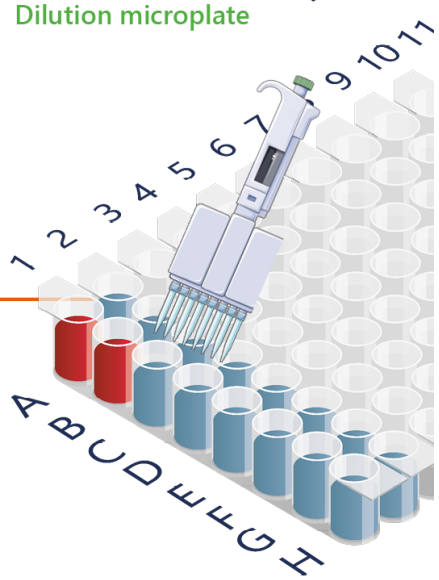
Mix




Dilution microplate

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

Room Temp. | 60 min | 3X Wash




Microplate of the kit*

**Breakable format for a precise adjustment to the number of samples*

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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