



MONOSCREEN^{Ab} ELISA

***E.coli* F5 (K99)**

ELISA kit for serodiagnosis of *E. coli* F5 attachment factor

Blocking test for blood sera and plasma

Diagnostic test for all species

Monowell

I - INTRODUCTION

Diarrhoea is one of the leading causes of death in calves in the first month of life. The enterotoxigenic *E. coli* bearing the F5 attachment factor can be found in less-than-three-day-old calves, especially in subjects that have not received any colostrum or receive colostrum that does not contain any antibody against this pathogen and its attachment factor. The antibodies that the cow produces in response to natural immunisation or vaccination are transmitted to her calf via the colostrum. The colostrum immunoglobulins frequently are not transmitted to the calves correctly (poor quality colostrum, late administration, too small an amount, pre-calving mastitis, etc.). As a result, the calf will be insufficiently protected from infection. The *E. coli* F5 ELISA KIT enables one to measure the suckling calf's specific protection against the F5 attachment factor of *E. coli*. For this, a serum sample must be taken in the first few days after birth when the calf is still protected by the colostrum and has not yet developed active immunity against the virus. However, you must wait at least 24 hours after the first dose of colostrum before taking the control blood sample to allow intestinal resorption of the immunoglobulins to take place. The kit may also be used to test the efficacy of vaccines.

As it is a blocking test, it can be used in all animal species.

II - PRINCIPLE OF THE TEST

The 96-well microplates have been sensitised by a monoclonal antibody specific for the F5 attachment factor of *E. coli*. An *E. coli* F5 + culture is then added to these microplates. The operator deposits the previously diluted test sera in the microplate's wells. After 2 hours' incubation and a rinse step, the operator adds the conjugate, which is a specific monoclonal antibody against *E. coli* F5 coupled to peroxidase. After incubating and washing the preparation, the operator adds the chromogen tetramethylbenzidine (TMB). This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. The intensity of the colour is inversely proportionate to the sample's serum titre. Positive and negative sera are provided with the kit to be able to validate the test results.

III - COMPOSITION OF THE KIT

- **Microplates:** 96-well microtitration plates. The entire surface of each microplate has been sensitised with *E. coli* F5
- **Washing solution:** One bottle of 20x concentrated washing solution. The solution crystallises spontaneously when cold. If only part of the solution is to be used, bring the bottle to 21°C +/- 3°C until disappearance of all crystals. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water. Store the diluted solution between +2°C and +8°C.
- **Dilution buffer:** One bottle of colored buffer for diluting samples and conjugate. The dilution buffer is ready to use. Store the solution between +2°C and +8°C. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.
- **Conjugate:** 1 bottle of anti-*E. coli* F5-peroxidase conjugate (horseradish peroxidase-labelled anti-*E. coli* F5 monoclonal antibody).
- **Positive serum:** 1 bottle containing the positive serum. Reconstitute this serum with 0.5 ml distilled or demineralised water. The reconstituted serum must be kept at -20°C. Divide this reagent into several portions before freezing it to avoid repeated freeze-thaw cycles. If these precautions are taken, the reagent may be kept for several months.
- **Negative serum:** 1 bottle containing the negative serum. Reconstitute this serum with 0.5 ml distilled or demineralised water. The reconstituted serum must be kept at -20°C. Divide this reagent into several portions before freezing it to avoid repeated freeze-thaw cycles. If these precautions are taken, the reagent may be kept for several months.
- **Single component TMB:** One bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C and +8°C protected from light. This solution is ready to use.
- **Stopping solution:** One bottle of the 1 M phosphoric acid stop solution.

	BIO K 295/1	BIO K 295/2
Microplates	1	2
Washing solution	1 X 100 ml (20 X)	1 X 100 ml (20 X)
Colored Dilution buffer	1 X 60 ml (1X)	1 X 60 ml (1X)
Conjugate	1 X 0,3 ml (50X)	1 X 0,5 ml (50X)
Positive serum	1 X 0,5 ml (1 X) freeze-dried	1 X 0,5 ml (1 X) freeze-dried
Negative serum	1 X 0,5 ml (1 X) freeze-dried	1 X 0,5 ml (1 X) freeze-dried
Single component TMB	1 X 12 ml (1 X)	1 X 25 ml (1 X)
Stopping solution	1 X 6 ml (1 X)	1 X 15 ml (1 X)

IV - ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

Distilled water, graduated cylinders, beakers, plastic tubes, tube rack, dispenser tips, reagent reservoir for multichannel pipettes, lid, adhesive for microplates, graduated automatic (mono- and multichannel) pipettes, microplate reader, and microplate washer and shaker (optional)

V - PRECAUTIONS FOR USE

- This test may be used for “in vitro” diagnosis only. It is strictly for veterinary use.
- The reagents must be kept between +2°C and +8°C. The reagents cannot be guaranteed if the shelf-life dates have expired or if they have not been kept under the conditions described in this insert.
- The concentrated wash solution may be stored at room temperature. Once diluted, this solution remains stable for six weeks if kept between +2°C and +8°C.
- Unused strips must be stored immediately in the aluminium envelope, taking care to keep the desiccant dry and the envelope's seal airtight. If these precautions are taken, the strips' activity can be conserved up to the kit's shelf-life date.
- Do not use reagents from other kits.
- The quality of the water used to prepare the various solutions is of the utmost importance. Do not use water that may contain oxidants (e.g., sodium hypochlorite) or heavy metal salts, as these substances can react with the chromogen.
- Discard all solutions contaminated with bacteria or fungi.
- The stop solution contains 1 M phosphoric acid. Handle it carefully.

- All materials and disposable equipment that come in contact with the samples must be considered potentially infectious and be disposed of in compliance with the legislation in force in the country.
- To guarantee the reliability of the results, one must follow the protocol to the letter. Special care must be taken in observing the incubation times and temperatures, as well as measuring the volumes and dilutions accurately.

VI – PROCEDURE

- 1- Bring all the reagents at 21°C +/- 3°C before use. Remove the microplate from its wrapper.
- 2- Dilute the concentrated washing solution 20-fold in distilled water. Be sure that all crystals have disappeared before dilution.
- 3- Dilute the blood sera twofold with the dilution buffer. Proceed in the same manner for the reference sera (positive and negative sera).
- 4- Distribute the dilute samples over the plate at the rate of 100 µl per well. Proceed in the same manner for the reference sera (positive and negative sera). Cover with a lid and incubate the plate at 37°C for 2 hours.
- 5- Rinse the plate with the washing solution prepared as instructed in the section “Composition of the Kit”. To do this, dispose of the microplate’s contents by flipping it sharply over a container filled with an inactivating agent. Let the microplate drain upside-down on a sheet of clean absorbent paper so as to eliminate all liquid. Add 300 µl of the washing solution, and then empty the plate once again by flipping it over above the containment vessel. Repeat the entire operation two more times, taking care to avoid the formation of bubbles in the microwells. After the plate has been washed three times proceed to the next step.
- 6- Dilute the conjugate 1:50 in the buffer for dilution (for example, for one plate dilute 250 µl of the conjugate stock solution in 12.25 ml of diluent). Add 100 µl of the dilute conjugate solution to each well. Cover with a lid and incubate the plate at 37°C for 1/2 hour.
- 7- Rinse the plate with the washing solution as instructed in step 5
- 8- Add 100 µl of the chromogen solution to each well on the plate. The chromogen solution must be absolutely colourless when it is pipetted into the wells. If a blue colour is visible, this means that the solution in the pipette has been contaminated.
- 9- Incubate for 10 minutes at 21°C +/- 3°C. This time is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.
- 10- Add 50 µl of stop solution per microwell. The blue colour will change into a yellow colour.
- 11- Read the optical densities in the microwells using a plate reader and a 450 nm filter. Results must be read fairly soon after the stopping solution has been added since the chromogen may crystallise in wells with strong signals and distort the results accordingly.

VII – CALCULATING THE RESULTS

Measure the optical densities of the positive and negative sera (OD pos and OD neg) and those of all the samples (OD samples).

Calculate the percent inhibition (% inhib) for each tested sample and the positive serum by means of the following formulas:

$$\% \text{ inh sample} = [(OD \text{ neg} - OD \text{ sample}) / OD \text{ neg}] * 100$$

$$\% \text{ inh positive} = [(OD \text{ neg} - OD \text{ pos}) / OD \text{ neg}] * 100$$

VIII – VALIDATING THE TEST

The test may be validated only if the following two conditions are met:

- OD neg – OD pos > 0.7
- % inh positive > 50%

IX – INTERPRETING THE RESULTS

Determine each sample's positivity using the scale shown in Table 1.

Table 1	Calculated value	Degree of positivity
	% inh < 20	0
	20 <= % inh < 40	+
	40 <= % inh < 60	++
	60 <= % inh < 80	+++
	80 <= % inh	++++

X – ORDERING INFORMATION

Monoscreen AbELISA *E.coli* F5 (K99)

1X 96 tests BIO K 295/1
2X 96 tests BIO K 295/2

