



ELISA kit for antigenic diagnosis of Rotavirus, E. coli F4, F5 attachment factors and Cryptosporidium

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<http://vacunek.com/shop/veterinaria/elisa-kit-for-antigenic-diagnosis-of-rotavirus-e-coli-f4-f5-attachment-factors-and-cryptosporidium/>

Product Summary

BIO K 307/2 o BIO K 307/5

Product Description

I - INTRODUCTION

Diarrhoea or scours in piglets can be common at both the neonatal and the post-weaning stage. It is a common cause of mortality in piglets and is often closely associated with poor hygiene, inappropriate husbandry (e.g. early weaning), stressful environment and inappropriate feeding factors. Piglet neonatal gastroenteritis is a multifactorial disease. It can be caused by viruses: TGE or rotavirus, by bacteria : E. coli (fimbrial strains) and Salmonella or by protozoan microorganisms such as Cryptosporidium. The diagnosis of the etiological agent of diarrhoea can only be performed in the laboratory because clinical signs do not allow to differentiate between the different microorganisms. The ELISA method is particularly useful when a large number of samples must be analyzed. The test is fast and reliable, and it can be evaluated at a glance if a spectrophotometer is not

available.

II - PRINCIPLE OF THE TEST

Specific antibodies produced against pathogens responsible of digestive diseases have been immobilized on alternate rows of 8 x 12-well microtitre plates. These antibodies allow the specific capture of the corresponding pathogens in the faecal samples. Rows A, C, E, G have been sensitised with these antibodies and rows B, D, F, H contain non-specific antibodies. These rows allow the differentiation between specific immunological reactions and non-specific binding so as to eliminate false positives. The faeces are diluted in dilution buffer and incubated on the microplate for 1 hour at 21°C +/- 3°C. After this first incubation step, the plate is washed, and then conjugates, peroxidase-labelled anti-pathogen monoclonal antibodies, are added to the wells. The plate is then incubated for 1 hour at 21°C +/- 3°C. After this second incubation step, the plate is washed again and the chromogen (tetramethylbenzidine) is added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If specific pathogens are present in the tested faeces, conjugates remain bound to the corresponding microwells and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titres of the specific pathogens in the sample. The enzymatic reaction can be stopped by acidification and the resulting optical density at 450 nm can be recorded using a photometer. The signals recorded for the negative control microwells are subtracted from the corresponding positive microwells optical densities. Positive control is provided with the kit so as to validate the test results.

III - COMPOSITION OF THE KIT

- Microplates: 96-well microtitration plates. The rows A, C, E, G are sensitised by specific antibodies, the rows B, D, F, H by non specific antibodies. Row A: anti-Rotavirus Row B: control Row C: anti- E. Coli F4 Row D: control Row E: anti-E. Coli F5 Row F: control Row G: anti-Cryptosporidium Row H: control - Washing

solution: bottle 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. - Dilution buffer: bottle colored and concentrated buffer for diluting samples. Dilute this concentrated dilution buffer 1:5 with distilled or demineralised water. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper. - Conjugates: vials of colored conjugates. These solutions are ready to use. Rotavirus (red), E. Coli F4 (yellow), E. Coli F5 (blue) and Cryptosporidium (green). - Positive Control: The reagent is ready to use. - Single component TMB: bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C and +8°C protected from light. This solution is ready to use. - Stopping solution: bottle of the 1 M phosphoric acid stop solution.