

Certificate of Analysis for NR-451

Bovine Coronavirus (BCoV), Mebus, Chemically Inactivated

Catalog No. NR-451

Product Description: Cell lysate and supernatant from human rectal tumor (HRT-18) cells infected with the Mebus strain of BCoV. The suspension of cell lysate and supernatant was treated with binary ethyleneimine to inactivate the virus.

Lot: 4462786 Manufacturing Date: 28AUG2005

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| Plaque Reduction Assay ¹ on HRT-18 Cells | Report results | 0 plaque forming units/mL |
| Cell Culture Immunofluorescence Assay ¹ on HRT-18 Cells | Report results | 0 fluorescent focus units/mL |
| Antigen-Capture ELISA ² | Report results | 1024 |
| Sterility (21-day incubation) Harpo's HTYE broth ³ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO ₂ | No growth | No growth |
| Mycoplasma Contamination Agar and broth culture (14-day incubation at 37°C) DNA Detection by PCR of Test Article nucleic acid | None detected None detected | None detected None detected |

Saif, L. J., R. A. Heckert, K. L. Miller, and M. Tarek. "Cell Culture Propagation of Bovine Coronavirus." <u>J. Tissue Culture Methods</u> 11 (1988): 139-146.

Date: 14 FEB 2006 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication

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²Titer is expressed as the reciprocal of the highest dilution with absorbance values greater than or equal to the mean absorbance of the mockinfected control plus three standard deviations and ratio between positive and negative coat greater than or equal to two. See, Cho, K. O., et al. "Evaluation of Concurrent Shedding of Bovine Coronavirus via the Respiratory Tract and Enteric Route in Feedlot Cattle." <u>Am. J. Vet. Res.</u> 62 (2001): 1436–1441. PubMed: 11560274.

³Àtlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.