

Certificate of Analysis for NR-869

Canine Coronavirus, UCD1, Chemically Inactivated

Catalog No. NR-869

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Product Description: Cell lysate and supernatant from canine tumor fibroblast (A-72) cells infected with the UCD1 strain of canine coronavirus (CCV). The suspension of cell lysate and supernatant was treated with binary ethyleneimine to inactivate the virus.

Lot: 4618585 Manufacturing Date: 18MAY2005

TEST	SPECIFICATIONS	RESULTS
Titer by TCID ₅₀ ¹ Assay ² in A-72 Cells	Report results	0 TCID ₅₀ /mL
Antigen-Capture ELISA ³	Report results	320
Sterility (21-day incubation) Harpo's HTYE broth ⁴ , 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

The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

Date: 20 JAN 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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²CCV was detected using an anti-porcine transmissible gastroenteritis virus serum based upon cross-reactions of these two viruses. After horseradish peroxidase conjugated secondary antibody, CCV-infected cells were identified with aminoethylcarbazole/hydrogen peroxide substrate development system. Only stained cells were considered as virus-infected.

³Titer is expressed as the reciprocal of the highest dilution that resulted in a mean absorbance greater than the mean absorbance of the mock-infected control plus three standard deviations.

⁴Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.