

**United States Department of Agriculture  
Animal and Plant Health Inspection Service  
Center for Veterinary Biologics  
P. O. Box 844  
Ames, IA 50010**

1. **Reagent Name:** *Escherichia coli* Anti-K99 Pilus Monoclonal Antibody (K99 MAb)
2. **Strain or Source:** Hybridoma 2BD4E4
3. **Lot Number:** IRP 557 (07)
4. **Fill Date:** May 18, 2007
5. **Expiration Date:** Not Applicable.

**Precautions:** There are no known hazards associated with this reagent.

6. **Intended Use:** For use in potency testing of *E. coli* biologicals containing the K99 antigen, according to **Supplemental Assay Method (SAM) 620**.
7. **Instructions for Use:** Dilute the K99 MAb 1:5,000 (or as optimized in specific titration studies) in cold carbonate-bicarbonate coating buffer (0.05M, pH 9.6) and use immediately according to **SAM 620**. Dilute to final dilution by adding 10  $\mu$ L undiluted MAb to 9.99 mL buffer (1:1000), then prepare final dilution by adding 2 mL (1:1000) to 8 mL buffer to prepare the 1:5000.
8. **Test of Reagent:** The K99 MAb was shown to be specific for K99 pilus antigen, and it demonstrated minimal nonspecific binding (background) in assays performed according to **SAM 620**.
9. **Container Size, Type, Weight, or Volume:** 150  $\mu$ L aliquots in 0.5-mL microfuge vials (sufficient for 12-15 plates).
10. **Storage Conditions:** -20° to -80°C for long-term storage. A vial may be held at 2°- 7°C for several months.
11. **CVB Technical Contact:** Bacteriology Section, Center for Veterinary Biologics, (515) 337-6140 or FAX (515) 337-7673.
12. **Origin and Passage History:** The hybridoma cell line secreting anti-K99 antibody was obtained from ATCC.

**13. Method of Preparation:** The hybridoma cell line was propagated in a bioreactor to produce supernatant fluid containing K99 MAb. The 500 mL harvest was originally frozen at -70°C or lower on December 23, 2002. The antibody was thawed in May of 2007; 75 mL was removed and aliquoted into 150 µL/vial. This lot was designated 2BD-001.

**14. Other:**

**Restrictions:** This reagent is to be used only in conjunction with biological potency testing according to SAM 620.

Reagent orders and feedback should be sent *including phone number* to the following email address: [CVB@aphis.usda.gov](mailto:CVB@aphis.usda.gov)

Reagent orders forms (APHIS 2018) are available from:  
[https://www.aphis.usda.gov/library/forms/pdf/APHIS\\_2018.pdf](https://www.aphis.usda.gov/library/forms/pdf/APHIS_2018.pdf)

**REVISED:** 18Apr14 alb