

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 629
4. **Fill Date:** December 12, 2013
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 1:2,000 (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**.
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:** 50  $\mu$ L aliquots in 0.5-mL microfuge vials.
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) 337-6100.
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 December of 2013; 35 mL was mixed with an equal part glycerol and aliquoted.

**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: [VS.DB.CVB.Reagent.Requests@usda.gov](mailto:VS.DB.CVB.Reagent.Requests@usda.gov)

Reagent orders forms (APHIS Form 2018) can be found on the CVB website.