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 Horseradish Peroxidase (HRP)-Labeled Monoclonal Antibody (K99-HRP Conjugate).

2. Strain or Source: Hybridoma 2BD4E4

3. Lot Number: IRP 595

4. Fill Date: December 14, 2010

5. Expiration Date: 30Apr22

Precautions: There are no known hazards associated with the use of this reagent.

6. Intended Use: For use in potency testing of *E. coli* biologicals containing the K99 pilus antigen, according to **Supplemental Assay Method (SAM) 620**.

7. Instructions for Use: Dilute the K99-HRP conjugate 1:550 in phosphate-buffered saline supplemented with 0.05% Tween 20 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**. The optimal use dilution was determined by titration, using the assay described in **SAM 620**.

9. Container Size, Type, Weight, or Volume: Approximately 50-µL aliquots in microfuge vials.

10. Storage Conditions: -20° to -80°C for long-term storage.

11. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6140 or FAX (515) 337-7673.

12. Origin and Passage History: Bioreactor fluid (Cell Lot #2BD-001) was prepared from the hybridoma cell line (2BD4E4) secreting anti-K99 antibody. The hybridoma was obtained from Molecular Genetics, Inc., Minnetonka, Minnesota.

13. Method of Preparation: Bioreactor fluid was conjugated with horseradish peroxidase using sodium periodate. The conjugated MAb was filter-sterilized through 0.22-µm filter unit, aliquoted, and stored at -70°C or lower.

14. Other:

Restrictions: 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.STAS.CVB.Reagent.Requests@aphis.usda.gov

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

REVISED: 29Mar18 tlt