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 680

4. Fill Date: Nov 27, 2020

5. Expiration Date: August 30, 2029

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 1:1100 in phosphate-buffered saline supplemented with 0.05% Tween 20 and use immediately, according to **SAM 620**.

8. Test of Reagent: The K99 monoclonal antibody (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) 337-6100.

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, and the conjugate was stabilized with sodium borohydride. The conjugated MAb was filter-sterilized through 0.45-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.DB.CVB.Reagent.Requests@usda.gov

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