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: *Clostridium tetani* HRP-labeled Monoclonal Antibody (Tetanus conjugate)

2. Strain or Source: Not applicable

3. Lot Number: IRP 458

4. Fill Date: February 21, 2001

5. Expiration Date: No expiration date has been assigned to IRP 458 because it has demonstrated over time to be stable if stored at -70°C or lower. The stability of this reagent will be routinely monitored by the Center for Veterinary Biologics.

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

6. Intended Use: IRP 458 serves as a standard conjugate when quantitating the titer of tetanus antitoxin by competitive enzyme-linked immunosorbent assay (CELISA).

7. Instructions for Use: Thaw, mix, and dispense approximately 30 µL of IRP 458 into screw-top or snap-top microcentrifuge tubes. Store at -70°C or lower. For use in the CELISA, add 20 µL of conjugate to 19.98 mL of conjugate diluent consisting of 15 mM NaH_2HPO_4, Na_2HPO_4, 145 mM NaCl, pH 7.2. Further dilute the conjugate to 1:25,000 by adding 1.0 mL of the 1:1,000 dilution to 24 mL of diluent.

8. Test of Reagent:

Toxin neutralization test - The biological activity of MAb 5C10, used to prepare IRP 458, was determined by conducting tetanus toxin neutralization tests in mice. MAb 5C10 provided protection against tetanic paralysis for 96 hours.

Isotyping of MAb 5C10 - The isotype of MAb 5C10 was determined to be IgG1 using an Isostrip isotyping kit (Boehringer Mannheim).

Reaction of MAb 5C10 to tetanus toxin C fragment - Binding of MAb 5C10 to the C fragment of tetanus toxin was determined by indirect ELISA. Absorbance values of 1.0 - 1.1 were recorded when 100 µL of MAB 5C10, diluted 1:160,000, were added to the well of a microtiter plate coated with purified tetanus toxin C fragment.

Sterility test – Five vials of IRP 458 were tested for sterility by inoculating the vial contents into tubes containing sterile fluid thioglycollate medium and soybean-casein digest medium. The tubes of inoculated medium were incubated at 20°C and 35°C for 14 days. No detectable growth appeared in any tubes of medium.

9. Container Size, Type, Weight, or Volume: Two-mL plastic screw-top microcentrifuge tubes containing 0.5 mL of conjugate.

10. Storage Conditions: Store IRP 458 at -70°C or lower.

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

12. Origin and Passage History: N/A

13. Method of Preparation: Anti-Tetanus MAb 5C10 was prepared by immunizing BALB/c mice with tetanus toxoid and purified tetanus toxin. Lymphocytes isolated from the spleen of an immunized mouse were fused with SP2/0 mouse myeloma cells. The MAb was produced by cultivating 5C10 hybridoma cells in a MiniPerm bioreactor containing DMEM with glucose, L-glutamine, and fetal bovine serum. The MAb was partially purified by ammonium sulfate precipitation, conjugated to horseradish peroxidase, and passed through a Millipore filter unit containing a 0.22-µm membrane. No preservative was added to the labeled MAb.

14. Other: None

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