VMRD, Inc.
PO Box 502, Pullman, WA 99163 USA

Telephone: + 1 (509) 334-5815
Fax: + 1 (509) 332-5356
E-mail: vmrd@vmrd.com
Web site: http://www.vmrd.com

Certificate of Analysis

CANINE ANTIBODIES TO SHEEP RED BLOOD CELLS

CATALOG NO.: 372-2
VOLUME: 1 ml
LOT: P050825-001-091507
EXPIRATION DATE: 15 September 2007

SUBAGGLUTINATION DOSE: 1:6

REAGENT: Canine Anti-Sheep Red Blood Cell Antibody

QUALITY CONTROL METHOD: Washed sheep red blood cells (SRBC) were sensitized with the reagent at the subagglutinating dose and tested as a positive control with Canine Coombs Reagent (catalog numbers 392-2 and 392-5; lot P0040907-001).

Specific Reaction: 3+ positive on sensitized SRBC; negative on unsensitized SRBC.

USES: 1) Positive control for Canine Coombs Test.
2) Hemagglutination test reagent for canine rheumatoid factor.

FOR IN VITRO LABORATORY ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

A. Determination of Antibody Dilution to Sensitize Sheep Red Blood Cells:

1. Make the dilutions of the canine antibody to sheep red blood cells that are above and below the dilution suggested by VMRD, Inc. The suggested dilution is found on the label of each reagent bottle. For instance, dilutions of Neat, 1:2, 1:4, 1:8, should be tested for antibody with the suggested dilution of 1:2. Make the dilutions with phosphate-buffered saline (PBS) or normal saline solution.
2. Add 0.1 ml of each dilution of antibody to 0.1 ml of 2% sheep red blood cells that have been washed three times.
3. Mix the tubes and incubate at 37°C for 30 minutes.
4. Centrifuge for 1 minute at 1500 x g.
5. To disassociate any nonspecific agglutination, hold each tube at a 45° angle and tap firmly on a table top 15 times just prior to Step 6.
6. Evaluate the contents of each tube by placing a small amount of the solution on a slide and viewing with a microscope (100X magnification is suitable).
7. The antibody solution that should be selected for sheep red blood cell sensitization is the lowest one which does not cause agglutination. This dilution is referred to as the subagglutinating dose.

B. Sensitization of Sheep Red Blood Cells:

1. Sheep blood collected in anticoagulant is centrifuged and the plasma removed.
2. The red blood cells should be washed 3 times in at least 5 volumes of PBS.
3. After final wash, make a 2% solution of sheep red blood cells in PBS.
4. Add an equal amount of the dilution of antibody determined in Step A (above) to be the subagglutinating dose and 2% sheep red blood cells.
5. Incubate for 30 minutes at 37°C.
6. Centrifuge and resuspend to one-half the total volume used in Step B-4 so that the final sensitized sheep red blood cell solution is 2%. If the resuspension is made in Alsever's solution, the sensitized cells can be stored for several days

C. Positive Control for Canine Coombs Test:

Use the 2% sheep red blood cells sensitized with canine antibody (Step B) as a positive control in the Coombs test by adding these sensitized cells to the Coombs reagent dilutions as described for the Coombs test. Sensitized cells should agglutinate when evaluated in the Coombs test with the canine Coombs reagent.

D. Reagent for Canine Rheumatoid Factor:

Procedure outlined in the references below should be followed for the canine rheumatoid factor test.

Precautions: Store at -10ºC until expiration date or at 4-8ºC if used within 6 months of receipt.

References:

Alexander, J.W. *et al.* Rheumatoid arthritis in the dog: Clinical diagnosis and management. *J. Am. Animal Hosp. Assoc.* 12:727-734 (1976).

Schulz, R. D. Laboratory diagnosis of immunologic disorders. Pages 453-463 in: *Current Veterinary Therapy, Volume 12*. Edited by RW. Kirk. W.B. Saunders Co., Philadelphia, PA (1995).