

CERTIFICATE OF ANALYSIS

CANINE ANTIBODIES TO SHEEP RED BLOOD CELLS

Catalog No.:	372-2
Volume:	1 ml
Lot:	P130325-002
Expiration:	10 April 2015
Reagent:	Canine Coombs Positive Control
Subagglutination Dose:	1/5

Description:

Canine Anti-Sheep Red Blood Cells with 0.09% sodium azide as a preservative.

Quality Control Method:

Washed sheep red blood cells (SRBC) were sensitized with the reagent at two fold dilutions above and below the subagglutinating dose and tested as a positive control with the Coombs Reagent (catalog number 392-2 or 392-5).

Specific Reaction: The subagglutinating dose for the SRBCs used for QC was determined to be 1/10. At this dilution there was a 2+ positive on the sensitized SRBCs at 1/2 of the Coombs Reagent.

Other Comments: The subagglutinating dose for this antiserum was determined to be 1/5. This dose may vary under your laboratory conditions, and especially with your source of sheep red blood cells. Therefore, we recommend that you titer this antiserum with your own SRBCs before using as your positive control. Please refer to Section A of the Coombs Positive Control procedure.

Uses:

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

Storage:

Store at < -10°C or at 2-7°C if used within 6 months of opening.

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).

Coombs Positive Control procedure

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

1. Make the dilutions of the 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 Coombs Test:

Use the 2% sheep red blood cells sensitized with 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 Coombs reagent.

D. Reagent for Rheumatoid Factor:

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