

Equine Coombs Positive Control

Equine anti-SRBC

Catalog No.:	472-2
Shelf Life:	2 years from date of qualification
Volume:	1 ml
Preservative:	0.09% Sodium Azide
Species of Origin:	Equine

Description:

Equine anti-sheep red blood cell antibody with 0.09% sodium azide as a preservative.

Intended Use:

1) Positive control for Equine Coombs Test.

Precautions:

The subagglutinating dose may also vary due to variation in sheep red blood cells. It is highly recommended that the subagglutinating dose be determined for each lot of sheep red blood cells (SRBC). The subagglutinating dose is lot specific see the COA.

Storage:

To avoid repeated freeze thaws store long term at < -10°C and short term (< 6 mo.) at 2-7°C.

References:

NA

Technical Data Sheet Version:

Version 1

Coombs Positive Control procedure

A. Determination of Subagglutinating Dose for Sensitization of 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.