MP Biomedicals, LLC

29525 Fountain Parkway
Solon, Ohio 44139

Telephone: 440/337-1200
Toll Free: 800/854-0530
Fax: 440/337-1180
mailto: biotech@mpbio.com
web: http://www.mpbio.com

TECHNICAL INFORMATION

ImmunO™

Catalog Number: 646451
Dog Anti-Sheep Red Blood Cells

Applications: Dog anti-sheep red blood cells can be used to sensitize sheep red blood cells (RBCs) for use in either a canine Rose-Waaler test [1-6], or in canine antiglobulin test as a positive control.

The Rose-Waaler test for rheumatoid factor was developed as a hemagglutination test in human medicine. A latex agglutination method was also developed. However, hemagglutination methodology is recommended for the canine rheumatoid factor assay since a latex test has not proven reliable[7].

The canine antiglobulin test is used to detect autoimmune hemolytic anemia and immune-mediated hemolytic anemia by hemagglutination. Since positive RBC specimens are not stable, they cannot be retained for use as positive controls. It is necessary for the veterinary testing lab to produce an artificial positive control.

In both applications, sheep RBCs are prepared by sensitization with sub-agglutinating doses of dog anti-sheep RBC. An approximate dilution of the antiserum is given for this purpose.

Reagent: Antiserum to sheep red blood cells is produced by immunization of dogs with sheep RBCs. The harvested antiserum is incubated at 60°C for 30 minutes to inactivate complement. The antiserum is tested to determine its agglutination titer and the sub-agglutination titer for sensitization of sheep RBCs in canine antiglobulin or Rose-Waaler procedures. The optimum dilution for sensitization of sheep RBCs in these procedures will vary from one laboratory to another. A sub-agglutinating dose is calculated for each lot of antiserum (see below). Please note that with some sheep RBC preparations, the sub-agglutinating dose may be as high as 1:500 p 1:2,000, even though we my report a much lower titer with our RBC preparation.

Working Dilution Determination: Lot specific - see lot specific credential

1.Select 12 x 75 mm test tubes.

2.Prepare several dilutions (0.1 ml/tube) dog anti-sheep RBC, bracketing the approximate dilution (sub-agglutinating dose) calculated for this lot of antiserum (e.g. if the reported dilution is 1:50, then prepare dilutions of 1:30, 1:40, 1:50, 1:60 and 1:70).

3.Add 0.1 ml of 2% washed, unsensitized sheep red blood cells to each tube (see "Preparation of Cells' steps 1 - 4).

4.Mix all tubes gently and incubate for 30 minutes at 37°C.

5.Gently resuspend the cells in each tube and examine the suspensions for obvious signs of agglutination. Setting patterns should not be used as a criteria for agglutination. Select the lowest dilution in which agglutination does not occur as the optimum working dilution for preparing sensitized cells with this lot of antiserum.

Preparation of Cells:

1.Collect sheep blood in Alsever's or another anticoagulant. Cells from 3 days to 2 weeks old are optimal for this procedure.

2.Centrifuge to separate RBCs and decant plasma.

3.Wash RBCs by suspending in 4 volumes of physiological saline, centrifuge and decant. Repeat two more times.

4.Suspend packed cells in 49 volumes of physiological saline to make a 2% RBC suspension.

5.Combine equal volumes of 2% RBCs and diluted dog anti-sheep RBC serum.

6.Incubate for 2 hours at room temperature, or 10 - 12 hours at 4°C.

7.Centrifuge and decant saline, resuspend in 4 volumes of physiological saline, centrifuge and decant saline.

8.Resuspend with 49 volumes of Alsever's solution (2% RBC). This sensitized cell preparation is stable for approximately one week at 4°C.

The Rose-Waaler Test for Canine Rheumatoid Factor: This test for rheumatoid factor (IgM antibodies to altered IgG) is based on the ability of the factor to agglutinate sensitized RBCs. The level of rheumatoid factor is indicated by the titer (dilution) at which agglutination occurs.

Specimen Collection: Collect specimens as serum and separate from erythrocytes. Incubate all specimens in a water bath at 56°C for 30 minutes to inactivate complement.

Controls: An EDTA plasma or serum (preferred) from a dog known not to have rheumatoid arthritis may be stored and used as a negative control. It is best to aliquot and freeze the negative controls for long-term storage. Canine Antiglobulin Reagent, code no. 64-635, may be used as a positive control. This reagent should produce positive agglutination to at least 1:128.

1.Select seven 12 x 75 mm test tubes for each specimen or control.

2.Pipette 0.1 ml of physiological saline into each tube.

3.Pipette 0.1 ml of specimen into the first tube (1:2) and mix.

4.Transfer 0.1 ml of the 1:2 dilution to the second tube (1:4) and mix. Repeat this procedure to the final tube (1:128).

5.Dilute the 2% sensitized sheep RBCs with an equal volume of saline.

6.Pipette 0.1 ml of 1% RBCs into each patient dilution and mix gently.

7.Incubate at 37°C for at least 1 hour, followed by 1 hour at room temperature.

8.Following incubation, observe each series of tubes of agglutination. Record the titer (highest dilution) at which agglutination occurs for each specimen.

Interpretation of Rose-Waaler Results: Titers of 1:2 or 1:4 are considered normal. A 1:16 titer or greater is positive. A titer of 1:8 may be considered suspect. Approximately 60% of dogs with other criteria of rheumatoid arthritis will test positive by this method.

Canine Antiglobulin Test: Sensitized sheep red blood cells can be used as an artificial positive control in the Canine Antiglobulin Test. The 2% sensitized suspension is simply introduced into parallel series of dilutions at the same point in the procedure that patient and negative cells are added. See the instructions for the Canine Antiglobulin Reagent.

Reference:

– Alexander, J.W., Beggs, S., Deuland, R., Schultz, R.D., **J. Am. Animal Hosp. Assoc., 12**, 727, 1976.
– Halliwell, R.E.W., Lavelli, R.B. and Butt, K.M., Canine rheumatoid arthritis - a review and case report, **ISAD 13**, 239, 1972.
– Newton, C.C., Lipowitz, A.J., Halliwell, R.E.W., et al., Rheumatoid arthritis in dogs. **JAVMA, 168**, 113, 1976.
– Rose, H.M., Ragan, C., Pearce, E. and Lipman, M.O. **Proc. Soc. Exper. Biol. and Med., 68**, 1, 1948.
– Schultz, R.D., Current Veterinary Therapy VI. Edited by Kirk, R.W. W.B. Saunders Co.: Philadelphia, Pa 1977.
– Schultz, R.D., and Adams, L.S., **Vet Clinic of N. America, 8**, 721, 1978.
– Wood, D.D., Hurvitz, A.A. and Schultz, R.D., **J. Vet. Immunol. and Immunopath, 1**, 103, 1980.

Note: This product may contain a preservative such as sodium azide, thimerosal or proclin. Please see lot specific chemical credential for preservative information.

If a titer/working dilution is not given above, please click here to see a general dilution chart for working with antibodies. Please note that the general dilution chart should only be used as a guideline. Each lab should determine their own optimal working dilution.

Will this antibody work with your application? Please click here to see a general chart of antibody applications. Please note that any information given above is primary application data. The general applications charts should only be used as a reference.