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ProductInformation

RED BLOOD CELLS SHEEP
(Glutaraldehyde Stabilized)

Product No. **R3378**

Store at 2-8 °C

This is a lyophilized preparation of glutaraldehyde fixed cells per modification of procedure of Bing *et al*.[1] These cells may be used in agglutination procedures but **NOT** in hemolytic procedures.

Reconstitute according to instructions on label using PBS, pH 7.2, and shake vial vigorously with a vortex mixer to obtain a smooth suspension of cells. The cells will settle and should be resuspended immediately prior to use. This preparation when reconstituted contains a trace of sodium azide* (approximately 0.03%) and bovine serum albumin (approximately 0.05%). To retard bacterial growth, you may incorporate 0.1% azide in your buffer when reconstituting and diluting the cell suspension for use.

***WARNING:** Sodium azide is toxic if ingested and may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.

PLEASE NOTE:
Since occasional clumping in stabilized red blood cell suspensions has been reported, Sigma recommends the following: After reconstituting and vortex mixing the 10% suspension, clumps may be dispersed by diluting the suspension to 1 or 2% (v/v) with PBS and passing it through a 22 to 24 gauge needle with a syringe 5 to 10 times. Alternatively a 100 or 500 µl Hamilton syringe may be used.

The cells may be washed to remove albumin if desired, tanned with 1/5000 or other concentrations of tannic acid, and sensitized with a variety of antigens or antibodies.

Storage
After reconstitution, nonsensitized cells may be stored at 4 °C for at least one month.
DO NOT FREEZE the cell suspension or its smoothness may be adversely affected.

Procedures

A. Procedure For Tanning Stabilized Red Blood Cells (SRBC)

1. To 1.0 ml of 10% SRBC at 37 °C add 1.0 ml of 1/5000 tannic acid solution, freshly prepared and prewarmed to 37 °C.
2. Incubate at 37 °C for 10 minutes.
3. Centrifuge 10 minutes at 1000 RCF; discard supernatant.
4. Wash cell cake with 40 ml PBS.
5. Resuspend tanned SRBC to 5% concentration with PBS, pH 6.4, or other buffer for use in sensitization.

B. Sensitization

1. To 1 volume of 5% tanned SRBC add 1 volume of solution containing 0.2 mg K-Globulin (Product No. G4386) per ml of PBS, pH 6.4.
2. Incubate (with stirring) for 30 minutes at 25 °C.
3. Centrifuge. Discard supernatant and wash packed cells with 100-500 volumes of PBS, pH 7.2. Resuspend the cells to 0.5-1% cell concentration with PBS, pH 7.2, containing 0.1% bovine serum albumin (BSA).

NOTE: For each sensitizing agent used, the temperature and length of incubation, concentration of sensitizing agent, buffer, and pH are important variables that may be empirically evaluated to obtain cells with optimal sensitivity. The reactivity of tanned sensitized cells may change with time.

C. Agglutination Procedure

A typical procedure for Anti-IgG agglutination using tanned stabilized red blood cells (SRBC) is as follows:

1. To each of the wells in two 8-well rows of a microtiter plate (U bottom) add 50 µl PBS, pH 7.2.

2. Prepare a fresh 1/1000 dilution (in PBS) of anti-human IgG (Product No. I9881). To the first well in each row add 50 µl of the diluted anti-IgG.

3. Prepare 2-fold serial dilutions by transferring 50 µL from the first well to the next well in the same row. Repeat the process through the 8 wells, discarding 50 µL from the last well after mixing. Do the same with the second row to obtain duplicate 2-fold serial dilutions.

4. To each well of the first row add 50 µL of the 0.5-1% sensitized cell suspension.

5. To each well of the second row add 50 µL of tanned only cells (not sensitized) diluted to 0.5-1% with PBS, pH 7.2, containing 0.1% BSA.

6. Mix the wells by gentle shaking. Cover the wells with plastic wrap.

7. Incubate the plate 60-90 minutes at room temperature. Do **not** disturb the plate during incubation.

8. Examine for agglutination against a white or lighted background to determine the resulting titer. If desired, plates may be stored in the refrigerator and read the following morning. Please refer to Rose and Friedman[2] for interpretation of agglutination patterns.

References

1. Bing D. H., Weyand J.M.G. and Stavitsky A. B.; Proc. Soc. Exp. Biol. Med., **124,** 1166, (1967)
2. Rose, N. R. and Friedman H., Eds., Manual of Clinical Immunology, 2nd ed. American Society for Microbiology, (1980)

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