RED BLOOD CELLS, CHICKEN (Glutaraldehyde Stabilized)
Product Number **R0504**
Storage Temperature 2-8 °C

ProductInformation

Product Description
Package size: 10 ml

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 but not in hemolytic procedures.

Preparation Instructions
Reconstitute according to instructions on label using PBS, pH 7.2 and shake vigorously, with vortex mixer, to obtain a smooth suspension of cells. The cells will settle and should be resuspended immediately before use. This preparation when reconstituted contains a trace of sodium azide* (approximately 0.03%) and bovine serum albumin (approximately 0.03%). 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.

Occasional clumping in stabilized red blood cell suspensions has been reported. After reconstituting and vortex mixing the 10% solution, diluting the suspension to 1 or 2% (v/v) may disperse clumps with PBS and passing in through a 22 to 24 gauge needle with a syringe 5 to 10 times. Alternatively a 100 to 500 µl positive displacement syringe may be used.

Storage/Stability
After reconstitution, nonsensitized cells may be stored at 2-8°C for at least one month. DO NOT FREEZE the cell suspension or its smoothness may be adversely affected.

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

Procedure

Procedure For Tanning Stabilized Red Blood Cells (SRBC)

1. To 1.0 ml of 10% SRBC at 25°C, add 1.0 ml of 1/5000 tannic acid solution, freshly prepared and prewarmed to 25°C.
2. Incubate at 25°C for 8 minutes.
3. Centrifuge 10 minutes at 1000 RCF; discard supenatant.
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.

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

Sensitization

1. To 1 volume of 5% tanned SRBC, add 1 volume of solution containing 0.2 mg γ-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.

Agglutination procedure

1. To each of the wells in two 8-well rows of microtiter plates (U bottom), add 50 µl PBS, pH 7.2. Some stabilized sensitized chicken cells may fail to agglutinate unless the ionic strength of the PBS is reduced to 0.1 to 0.2X the normal concentration.
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. Cover cells with plastic wrap. Mix the wells by gentle shaking.
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 for interpretation of agglutination patterns.

References

1. Bing D.H., et al. Proc. Soc. Exp. Biol. Med. **124**:1166-1170 (1967)
2. Rose N.R. and Friedman, H. (Editors), Manual of Clinical Immunology, 2nd ed. American Society of Microbiology, 1980

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