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ProductInformation

LARCOLL
Product Number **L 0650**
Technical Bulletin No. ARA-100

TECHNICAL BULLETIN

Product Description

Corash and coworkers[1] in the early seventies purified a crude form of arabinogalactan for use in density gradient separation of red blood cells. Many researchers have since found arabinogalactan to be indispensable in their work in cell separations. The availability of Larcoll (Product No. L 0650) has eliminated the time-consuming purification of crude arabinoglactan.

Larcoll is an ultra-refined arabinogalactan prepared specifically for density gradient separations of cells, viruses and organelles. It is nontoxic, completely compatible with biological materials, readily soluble in water and can be sterilized by microfiltration or autoclaving. Solutions of Larcoll have low viscosities and ideal densities. They are not sensitive to changes in pH or dissolved salts and are readily adjusted to the required osmolarity. The power is stable indefinitely. Stock solutions can be stored for at least one year at –20 °C.

For anyone not familiar with the use of arabinogalactan (Larcoll) in density gradient separation of cell populations, it is strongly recommended that pages 94-104 of Chapter 8, Volume 16, Methods of Hematology by L. M. Corash,[2] be studied.

Preparation Instructions

Larcoll-BSA Solution

1. Into a weighed, 1-liter glass beaker add 400 mL water. Heat the water to a gentle boil.
2. Add 250 g Larcoll with stirring, and gently boil for at least 30 minutes. Cool the solution to room temperature and dilute to 834 g with deionized water.
3. Add with stirring:
   23.5 g bovine serum albumin (BSA)
   1.25 g glucose
   0.725 g $MgCl_2.H_2O$
   41.3 mL 0.3 M K_2PO_4- buffer, pH 7.4
4. Adjust to pH 7.4 with 1 N NaOH.
5. Determine the osmolarity and adjust to 290 mosm/L with NaCl.
6. Measure the density of the final solution by using a 500 µL micropipet as a pycnometer.

BSG-BSA SOLUTION (Buffered Saline with Glucose)

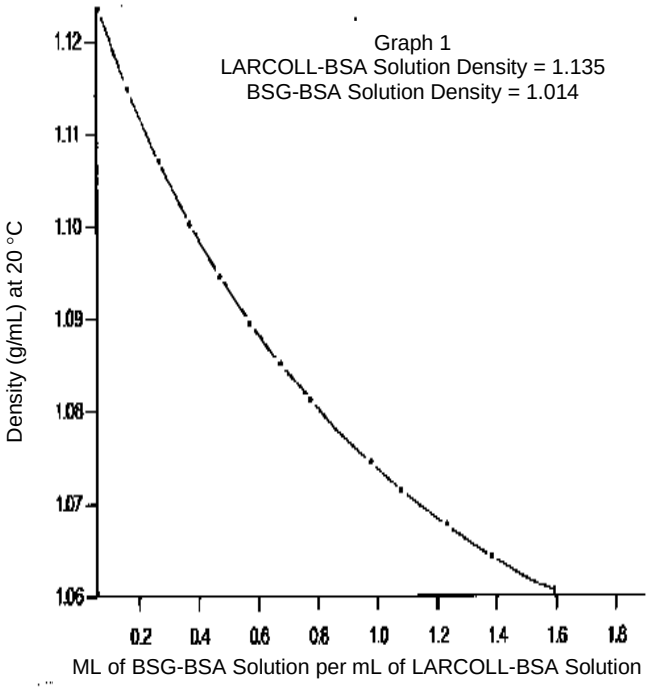
1. To 600 mL deionized water, add with stirring:
   4.86 g NaCl
   0.732 g Na_2HPO_4
   0.131 g NaH_2PO_4
   1.20 g glucose
   18.0 g BSA
2. Adjust to pH 7.4 with 1 N NaOH.
3. Determine the osmolarity and, if necessary, adjust to 290 mosm/L with NaCl.
4. Measure the density of the final solution by using a 500 µL micropipet as a pycnometer.

Storage of solutions

Filter the solutions through a 0.45 µ filter and store in 50 mL aliquots in capped containers at –20 °C. Solutions are stable for at lease one year if kept frozen.

Density Adjustment

Solutions with appropriate densities can e prepared by mixing calculated amounts of LARCOLL-BSA Solution and BSG-BSA Solution (see Graph 1).



Procedure

A. Separation of red blood cells on Larcoll gradients[4]

1. Chill rotor and buckets.
   a) For 1-3 ml of cells, use SW 50.1 rotor.
   b) For 3-6 mL of cells, use SW 27 rotor.
   c) For 6-10 mL of cells, use SW 27 rotor with 35 mL buckets.
2. Prepare gradients in clear centrifuge tubes. Thaw appropriate Larcoll-BSA Solution and BSG-BSA Solution.
3. For discontinuous layers, carefully allow solutions to flow from a 5 mL pipet down the side of the tube.
   a. For 5 mL centrifuge tubes, use 1 mL of the proper ratio of Larcoll-BSA Solution and BSG-BSA Solution (see Graph 1) per layer on 0.5 mL of a dense cushion (about 1.11 g/mL). Use a smaller volume per layer if more layers are required.
   b. For 17 mL tubes, use 2.5 mL of the proper mixture of Larcoll-BSA Solution and BSG-BSAA Solution per layer on top of a 0.75 mL cushioning layer
4. For continuous gradients, use a linear gradient forming device. Put 1/2 the desired gradient volume into each chamber, spanning the desired density range with overlap of the extreme densities by about 0.004.
   a. Make the gradients by referring to Graph 1. For example, 1.5 mL of BSG-BSA Solutions plus 1 mL of Larcoll-BSA Solution (density = 1.0624) in the low density chamber and 0.3 mL BSA-BSA Solution plus 1 mL Larcoll-BSA Solution (density = 1.1071) in the high density chamber for separation of reticulocytes from normal blood might be used. For irreversibly sickled cell separations, 1.1 mL BSG-BSA Solution plus 1.0 mL Larcoll-BSA Solution (density = 1.124) could be employed.
   b. Form gradients one at a time, running the proper mix of Larcoll-BSA Solution and BSG-BSA Solution on top of a high density cushion. Make sure the flow does not get ahead of the mixing of the two solutions.

B. Procedure

1. Resuspend the packed cells (from which the WBC have been removed by cellulose filtration) to no more than 30% hematocrit.
2. Layer the cell suspensions on top of the gradients with a Pasteur pipet. If cells are scarce, rinse the tubes carefully to get maximal transfer.
3. Make sure the tubes are filled to within 1/8" of the top, then balance them to within 0.1 g of each other by adding BSG-BSA Solution.

   NOTE: If two different density ranges are being assayed, balance the low density tubes first. If the higher density tubes first. If the higher density tubes are balanced first. there may not be enough room in the tube to bring the low density tube up to the desired weight.

4. Carefully clean the outside of the tubes to avoid having them stick in the buckets.
5. Spin samples at 20,000 rpm (SW 27 rotor) or 25,000 rpm (SW 50.1 rotor) for 45 minutes (30 minutes if time is short).
6. Sketch a diagram of the gradients.
7. Remove the cells from the tubes with a Pasteur pipet. Run the pipet around the edge of the tubes at the top of the solution to collect all of the cells at a given level. Place the cells into graduated centrifuge tubes.

   NOTE: Collecting the cells through a hole pierced in the bottom of the tube does not work because cells tend to stick to the wall of the tube.

8. To wash the cells by sedimentation, dilute the cell suspensions with BSG-BSA Solution. Cover with Parafilm® and invert to mix well. Spin the cell suspensions at 2800 rpm for 5 minutes and aspirate to remove the supernatant liquid.
9. Resuspend the cells in BSG-BSA Solution. Pool the corresponding layers if there is more than one tube for a given cell population. Wash one or more times with BSG-BSA Solution. Pool the corresponding layers if there is more than one tube for a given cell population. Wash one or more times with BSG-BSA Solution. Centrifuge at 2000 rpm for 5 minutes after each wash.
10. Record the packed volume of each cell population and make up to appropriate hematocrit for subsequent experiments.

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References

The literature references cited below indicate a few of the many uses of arabinogalactan in cell research.

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