BulkLysis™

Ref:CYT-BL

RUO

For Research Use Only. Not for use in diagnostic procedures

INTENDED USE

BulkLysis™ is an hypotonic lysing solution which provides osmotic lysis of the erithrocytes **before** immunofluorescence staining of peripheral blood or bone marrow aspirate samples.

SUMMARY AND EXPLANATION

The advanced multiparameter tools for the immunophenotypic characterization of different cell subsets reffers not only to the instruments, but also to the reagents and detection limit (sensibility). In order to achieve great sensibility, starting with higher cell numbers is recommended. This can be achieved by using a fixative-free erithrocyte lysing solution before staining and that does not interfere with immunofluorescence cell staining.

REAGENT PROVIDED

BulkLysis™ is provided as 100 ml 10x concentrated solution containing ammonium chloride, potassium hydrogen carbonate, sodium azide (0,09% (m/v)) and EDTA. This volume is sufficient for 20 tests (5 ml **BulkLysis™** per test, for lysing each 2ml of sample). Reagent is not considered sterile.

STORAGE CONDITIONS

Concentrated **BulkLysis™** is stable until the expiration date shown on the label when it is stored at 2-8 ◦C. The pH of the reagent may increase during unsuitable storage, which may affect cells scattered light in the FSC/SSC dot plot. The pH of the reagent should be between 7 and 7,4. If the pH is out this range, it should be adjusted by adding diluted solutions of HCl or NaOH.

WARNINGS AND RECOMMENDATIONS

1. The reagent is stable until the expiration date shown on the label if it is properly stored. Do no use after the expiration date shown on the label. If the reagents are stored in conditions different from those recommended, such conditions must be validated by the user.
2. Alteration in the appearance of the reagent, such as the precipitation or discoloration indicates instability or deterioration. In such cases, the reagent should not be used.
3. It is recommended handling the product with appropriate protective gloves and clothing, and its use by personnel sufficiently qualified for the procedures described. Avoid contact of samples with skin and mucous membranes. After contact with skin, wash immediately with plenty of water.
4. Use of the reagent with incubation times or temperatures different from those recommended may cause erroneous results. Any such changes must be validated by the user.
5. Any serious incident relating to the product must be reported to Cytognos S.L. as well as the competent professional authority of the Member State in which the user is established.

PROTOCOL

1. Dilute the concentrated BulkLysis™ solution (1:10) using distilled water.
2. Transfer no more than 2 ml of the sample containing at least 10 x 10^6 nucleated cells (peripheral blood or bone marrow aspirate collected on anticoagulant) to a 50 ml tube.
3. Fill the tube up to reach 50 ml volume using the diluted BulkLysis™.
4. Shake and incubate for 15 minutes on a laboratory roller mixer.
5. Centrifuge for 10 minutes at 800 xg speed.
6. Gently discard the supernatant using a vacuum pump or Pasteur pipette.
7. Add 2 ml of PBS + 0,5% (m/v) BSA + 0,09% (m/v) Sodium Azide and resuspend the cellular pellet. Fill up to 50 ml with the same washing buffer.

Note: In case you have a significant cell concentration, it is important to re-suspend the cell pellet with 2 ml PBS + 0,5% (m/v) BSA + 0,09% (m/v) Sodium Azide by mixing slowly and gently. That is, add the PBS slowly, little by little, and mix gently between lots. If it is necessary, you can re-suspend by using a pipette. Then, add PBS + 0,5% (m/v) BSA + 0,09% (m/v) Sodium Azide up to 50 ml. This should help in avoiding clumping.

8. Centrifuge for 5 minutes at 800 xg speed.
9. Gently discard the supernatant using a vacuum pump or Pasteur pipette.
10. Add 2 ml of PBS + 0,5% (m/v) BSA + 0,09% (m/v) Sodium Azide and resuspend the cellular pellet. Pass the cellular suspension into a 5ml flow cytometry tube. Add 2 ml of washing buffer into the 50 ml tube, mix it well and transfer to the 5ml tube.

Note: In case you have a significant cell concentration, it is important to re-suspend the cell pellet with 2 ml PBS + 0,5% (m/v) BSA + 0,09% (m/v) Sodium Azide by mixing slowly and gently. That is, add the PBS slowly, little by little, and mix gently between lots. If it is necessary, you can re-suspend by using a pipette. This should help in avoiding clumping.

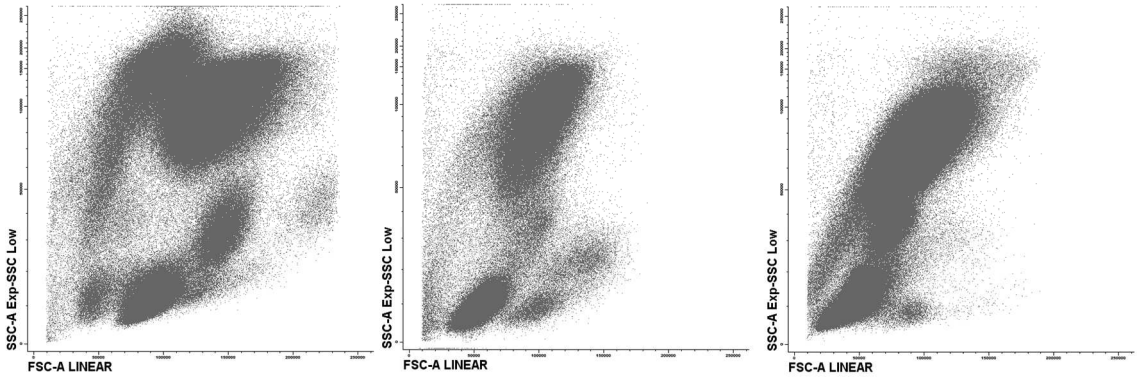
11. Centrifuge for 5 minutes at 540 xg.
12. Discard the supernatant and resuspend the cellular pellet adjusting the volume in order to obtain 1x10^5 cells/µl.
13. Transfer the cells into an appropriate tube for staining procedures.

For more informations about the protocol please enter the EuroFlow website.

FLOW CYTOMETRY ANALYSIS

Check that the cytometer is correctly aligned and standardized for light dispersion and that the right compensation has been set following the instructions for each cytometer.

The figure bellow shows representative flow cytometry data on normal peripheral blood treated with BulkLysis™ only (prior to staining), other commercial lysis with fixative agent (after staining) and the combination of the two (BulkLysis™ prior to staining and fixative agent lysis after staining). This reagent sometimes separates the leukocyte subpopulations into two discrete populations with the same SSC characteristics, but different FSC (it has to be taken into account whenever specific population acquisition gates are to be used). The forward/side scatter characteristics might change compared to the classic image due to the lack of the fixative agent. In order to obtain the same FSC/SSC image as for whole blood normal stainings it is advisable to use a fixative lysing solution, after the incubation with the monoclonal antibodies.



BulkLysis | Lysis with fixative agent | BulkLysis + lysis with fixative agent

Figure: Scatter characteristics of peripheral blood lysed with BulkLysis™ working solution and/or lysis with fixative agent (FSC threshold at 10 000; Image obtained using Infinicyt ® software, Cytognos, Spain).

Troubleshooting: In general, after the BulkLysis™ with ammonium chloride the cell pellet obtained should be free of red blood cells. In some cases, it was observed a red cell pellet following the first centrifugation of the protocol, due to: the medical treatment status and type and/or the age of the sample from its extraction untill its processing, but also related to deteriorated lysis solution (pH, below 20◦C).

WARRANTY

This product is warranted only to conform to the quantity and contents stated on the label. There are no warranties that extend beyond the description on the label of the product. Cytognos's sole liability is limited to either replacement of the product or refund of the purchase price.

EXPLANATION OF SYMBOLS

	Use by (YYYY-MM)
	Storage temperature limitation
	Keep out of sunlight
	Consult instructions for use
RUO	For research use only
LOT	Batch code
REF	Catalogue number
	Manufacter

PRODUCED BY

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