

TITLE: LYSING REAGENT

PRINCIPLES OF THE PROCEDURE:

This reagent is used for the lysing of Red Blood Cells in samples for Flow Cytometry testing.

SPECIMEN REQUIREMENTS: NA

REAGENTS/SUPPLIES:

8.26 g Ammonium Chloride (NH₄Cl) -- order from Sigma A-5666

1.0 g Potassium Bicarbonate (KHCO₃) -- order from Sigma P-4913

0.037 g Tetra Sodium EDTA -- order from Sigma ED4SS

NOTE: All of the above chemicals are stored at room temperature and can be found in the Tissue Culture/Research Lab Room #624.

EQUIPMENT & INSTRUMENTATION:

Weigh boats

Scale

PROCEDURE:

Measure carefully the above chemicals.

Pour into a bottle (must be at least 1000 ml).

Add 1000 ml double distilled water, cap and mix to dissolve.

For best results keep the solution in a water bath at 37°C.

Discard after 24 hours.

Do not pH.

PROCEDURAL NOTES:

IN THE CLINICAL LAB MAKE FRESH DAILY.

QUALITY CONTROL GUIDELINES:

This reagent must be checked for lysing ability prior to use on patient samples as stated below. In this procedure, the amount of time it takes to lyse red cells is measured. To be acceptable, red cell lysis must be complete within 3 minutes of the addition of the reagent.

Pipet 50 µl whole blood from the daily "healthy donor" sample into a labeled tube. Add 3.5 ml lysing reagent to this tube and cap.

Invert to mix. Immediately after adding the lysing reagent to the tube, start timing for 3 minutes.

To check for completeness of the red cell lysing, hold the tube up to a printed page. When the print can be read through the tube, the lysis is complete.

Document that this procedure has been completed on the LYSING REAGENT CHECK LIST. A sample is on the following page.

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		Date	Date	Date	Date	Date	Date
REVISED BY		Sjs	aec				

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		Date	Date	Date	Date	Date	Date
APPROVED BY		Ccs					