

TITLE: Molecular Probes Live/Dead Green Dye Protocol Viability for Clinical Samples

PRINCIPAL OF THE TEST:

Too differentially stain live and dead cells. This assay is based on a reaction of a fluorescent reactive dye with cellular amines. The reactive dye can permeate the membranes of necrotic cells and react with free amines both in the interior and on the cell surface, resulting in intense fluorescent staining. In contrast, only the cell surface amines of viable cells are available to react with the dye, resulting in relatively dim staining.

This method will better enable us to determine if large populations of dead cells exist in our samples.

SPECIMEN REQUIREMENTS:

BONE MARROW or WHOLE BLOOD collected in Sodium Heparin or EDTA tube. Keep at room temperature. Specimens have to be processed within 48 hours of the time of collection.

CSF, PLEURAL FLUID AND BRONCHIAL WASHES, collected in sterile containers. Keep at room temperature. Specimens have to be processed within 6 hours of time collected.

TISSUE/LYMPHNODES SPECIMENS should be sterilely minced using a scalpel. Cut into sections from 0.01 to 0.25 cubic cm in size. They should be placed into a screw top, leak proof container with RPMI 1640 or alternatively HANKS Balanced Salt Solution.

NOTE: both should be supplemented with Gentamicin sulfate, 50ug/ml, to retard bacterial growth.

Keep specimens on ice or at 4°C. . Specimens have to be processed within 12 hours of collection. Samples with viability of less than 85% will not be reported

ALL REQUISITIONS MUST INDICATE:

- a) **PATIENT NAME AND OR ID NUMBER.**
- b) **DAY AND TIME OF COLLECTION.**

REAGENTS AND PREPARATION:

The Live/Dead Dye will be prepared for a weeks worth of test (i.e. 100 tests/week.) The working stock will be prepared at a 1:50 dilution in PBS. The samples will receive 5ul of the working stock. This working stock solution should be stored in the refrigerator (4-8°C), in the dark for no longer than 7 days.

Reagents:

LIVE/DEAD® Fixable Green Dead Cell Stain Kit for flow Cytometry (Invitrogen Molecular Probes cat # L-23101). To prepare stock solution bring one vial of the supplied fluorescent reactive dye (Component A) and the one vial of anhydrous DMSO (Component B) to room temperature before removing the caps. Add 50 µL of DMSO to the vial of reactive dye. Mix well and visually confirm that all of the dye has gone into solution. As soon as possible, aliquot the reconstituted stock solution into 10 ul aliquots

Working Stock preparation:

Remove an aliquot for the Live Dead Green from the Clinical Lab freezer.
Add 490 ul of PBS to make a 1:50 dilution. This is enough for 100 tests.
Date and initial the vial.

This will be stored in the Clinical Lab refrigerator, in the 4 color panel box, for up to 1 week.

Use 5ul per test.

EQUIPMENT & INSTRUMENTATION: NA

PROCEDURE:

1. Add 5ul of the 1:50 dilution working stock solution to the appropriate tubes. This solution will be placed in the Clinical Lab Refrigerator with the 4-Color Antibodies.
2. Add the appropriate volume of sample to the tube and vortex.
3. Incubate the tube on ice in the dark for 20-30 minutes.
4. Vortex the sample after the incubation period has is done.
5. Add 3.5mls of the Lyse Reagent and invert the tube to mix thoroughly.
6. Let the tube stand for 5 minutes.
7. Centrifuge the tube at 3200 rpm for 3 minutes.
8. Decant and blot tube.
9. Add 3.5mls of PBS to wash.
10. Centrifuge the tube again at 3200 rpm for 3 minutes.
11. Decant and blot tube.
12. Add 0.3ml-0.5ml of 2% Formaldehyde to fix the sample and place in the refrigerator until acquisition.

PROCEDURAL NOTES: None

QUALITY CONTROL GUIDELINES: Blood from a Healthy Donor should be stained daily (excluding weekends) with the Live Dead Green dye to confirm that it is working properly. As a new lot of Reagent is prepared it should be tested with that day's healthy donor or similar sample in parallel with current lot to confirm that both give comparable results.

EXPECTED RESULTS: Samples with viability less that 85% should be reported out as unacceptable.

REPORTING RESULTS & CALCULATIONS: Each sample run in the Clinical Laboratory will have this test run. The results will be included in the report.

REFERENCES:

INVITROGEN MOLECULAR PROBES LIVE/DEAD KIT INSERT.

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APPROVED BY		Pkw					