

AO/EB or AO/PI for Viability

(Mario Roderer – 6/02)

Reagents:

- 1 μ l Acridine Orange Stock (5 mg/ml)
- 1 μ l Ethidium Bromide Stock (3 mg/ml)
- 1 ml PBS

Method:

AO-EB Working Solution

- 1) Add AO and EB to PBS and mix well.
- 2) Store at room temperature for up to 2 weeks.

To Use:

- 1) Dilute cells with equal volume of AO-EB working solution.
- 2) Immediately look at cells under fluorescence microscope.

Red Cells = Dead
Green Cells = Live

Notes from Mario Roderer:

This is a fabulous way to do viability testing! Once you do this method, you will never do a trypan blue (yech) again. I learned to do this in the Herzenberg laboratory at Stanford, brought it to the VRC--and we've now incorporated it in our clinical trials. Every time we thaw PBMC for doing immune function assays, we assess the viability by fluorescence first (and, in fact, if viability is below a threshold, I think 60%, we discard the sample). We've even developed an SOP for it.

We use a combination of acridine orange and ethidium bromide (not PI)--under a fluorescence scope, "green" is live and "red" is dead--no ifs, ands, or buts--and easily scored by even the most green students with risking a red face.

In any case, our procedure is to prepare 3 mg/ml ethidium bromide in absolute ethanol and 5 mg/ml acridine orange in ethanol. Store this stock in a dark vial, refrigerated. To make a working solution, take 1 microliter of each added to 1 milliliter of PBS. This we store at room temp by the fluorescence microscope, and make fresh every few weeks. Please note that AO and EB are considered highly carcinogenic: use gloves and a face mask when preparing the concentrated stock solution, and use gloves when handling the working solution.

Dilute cells with an equal volume of the working solution and immediately look on the fluorescence microscope (you can also dilute 1:10 if the cell count is too high). Remember to take this dilution into account when you calculate original numbers.

Mario Roderer

(PS, if you don't have EB, you could probably use PI at the same concentration in its place.

