

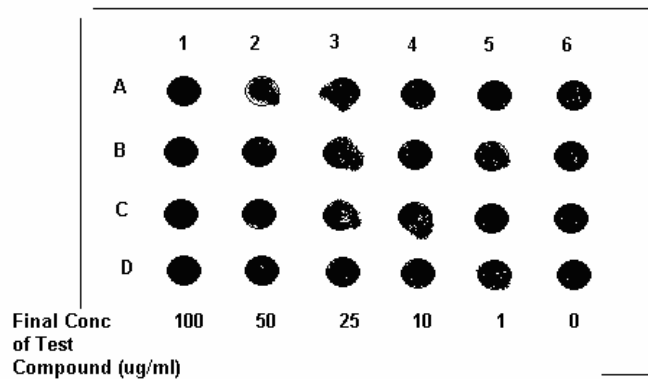
## Procedure for the treatment of NIH 3T3 cells with test compounds:

### Materials:

Test Material: Molday ION Rhodamine B (2mg Fe/ml), Catalog No. CL-50Q02-6A-50

### Procedure:

- 1.) **NIH 3T3 cells** are seeded into a suitable multiwell plate such as 24-multiwell plate 1-2 days before the day of the experimental procedure and grown to a sub-confluent state of approximately 70-80% confluence on the day of the experiment.
- 2.) **Preparation of test material.** On the day of the experiment prepare a sufficient amount of the test material by diluting test material in medium to a concentration of 100 ug Fe/ml, 50 ug Fe/ml, 20 ug Fe/ml, and 2 ug Fe/ml. The concentration of the test material is now 2X of the desired concentration needed to be tested.
- 3.) Prior to adding test material, replace the cell medium with fresh medium to  $\frac{1}{2}$  the normal working level.
- 4.) Add the freshly prepared 2X test material/medium solution (step 2) to the cells as indicated by the figure. The volume added should be equal to the volume of medium added in Step 3..



- 5.) Place the cells into the incubator for 4 to 20 hours dependent upon the degree of labeling desired. Proceed to cell fixation by treating 4 wells of cells at a time through the fixation process (A1-D1, A2-D2, etc.) if desired. Otherwise remove the medium and replace with fresh culturing medium. (In the case of cell fixation, fresh medium does not need to be added. The cells are washed with PBS with Mg and Ca, and subsequently fixed within several minutes of processing.
- 6.) Allow a suitable time for the cells to recover before further processing or imaging.

### SUPPLEMENTAL INFORMATION:

The concentration used to treat the cells should be a maximum of 100ug Fe/ml, if the material is supplied at a concentration of 2mg Fe/ml, this requires a 1:10 dilution to make the 2X mixture, with the subsequent 1:2 dilution when added to the cells yielding the 100ug Fe/ml maximum concentration. The cells can also be treated with a 1ug Fe/ml solution or less with noticeable loading still occurring.