

**Acridinium Ester Labeling Worksheet**  
**to be used with Enzo Life Sciences Catalog No. ADI-907-001**

Calculations:

1. Amount of Material to be Labeled: \_\_\_\_\_ mg
2. Molecular Weight of Material: \_\_\_\_\_ gms/mole ( i.e. daltons)
3. Moles of Material to be Labeled:  

$$\frac{\text{Amt. from \#1}}{\text{MW from \#2} \times 1,000} = \frac{\text{_____ mg}}{\text{(_____ gms/mole} \times 1,000)} = \text{_____ moles}$$
4. Ratio of Acridinium Ester (A<sup>+</sup>) to be added (we suggest 1 to 5 fold): \_\_\_\_\_
5. Moles of Acridinium Ester to be added:  
 moles from #3 x Ratio from #4 = \_\_\_\_\_ moles x \_\_\_\_\_ X = \_\_\_\_\_ moles of A<sup>+</sup>
6.  $\mu$ gs of Acridinium Ester needed:  
 moles from #5 x  $6.3255 \times 10^8$  = \_\_\_\_\_ moles x  $6.3255 \times 10^8$  = \_\_\_\_\_  $\mu$ g of A<sup>+</sup>
7.  $\mu$ Ls of Acridinium Ester to be added to the Material to be labeled:  

$$\frac{\mu\text{gs from \#6}}{2 \mu\text{g}/\mu\text{L}} = \frac{\text{_____ } \mu\text{g}}{2 \mu\text{g}/\mu\text{L}} = \text{_____ } \mu\text{L of A}^+$$

Labeling Flow:

1. Pipet the Material to be labeled into a test tube. Add a micro stirbar.
2. Add the amount of Acridinium Ester solution (\_\_\_\_\_  $\mu$ L of A<sup>+</sup> Solution, from Step 7 above) to the Material to be labeled. Vortex. Stir the solution at room temperature for 30 minutes.
3. Add 10 $\mu$ L of the 10% lysine solution to the tube, vortex.
4. Stir the solution at room temperature for 15 minutes.
5. Apply the labeled material to the prepared column and collect the eluant into tube 1.
6. Add 800 $\mu$ L of column buffer to the tube which contained the labeled material, vortex, and apply this solution to the column and collect this eluant also into tube 1.
7. Adding 1mL at a time, apply column buffer and collect the fractions into separate tubes for a total of 12-14 tubes.
8. Pipet 1 $\mu$ L from each tube into duplicate tubes (or wells) and read in a luminometer.
9. After plotting the column profile, pool the fractions selected, vortex, aliquot and store long term at -20°C.