Title: Procedural Worksheet for Preparation of FAE Reagent

Laboratorian: __________________________
Date of Preparation: ____________________
Lot of FAE Concentrate: __________________
Lot Expiration Date: _____________________
Date of Buffer: _________________________
Volume Made: __________________________

Procedure: Check off the following steps in FAE preparation as completed:

___ 1. Obtain required volume of filtered FAE Reagent Buffer from the A&R Core. (Two or two and a half liters is a convenient volume.)

___ 2. Verify that the FAE concentrate has been filtered through a 0.2um filter.

___ 3. Thoroughly mix the FAE concentrate by swirling for 2 minutes.

___ 4. Prepare a 1:100 dilution of the FAE concentrate by pipetting 10uL concentrate into 990 mL of FAE reagent Buffer.

___ 5. On ACS:180 A8334 or A1163 run 25uL totals in 5 replicates on the 1:100 FAE. Do this by editing the TA Test Definition, changing from 2 replicates to 5 on the first page and from 10uL sample volume to 25uL on the second page. Remember when finished to reset the T-Def to the original settings. Record the totals and mean total below.

          RLU for 25uL 1:100 FAE: _______________  Mean: _______________
          _______________
          _______________
          _______________

___ 6. Calculate the RLU for 25uL of the FAE concentrate by multiplying the mean for 1:100 FAE by 100 and record below.

          RLU for 25uL FAE concentrate: _______________

___ 7. Determine and add 80% of the FAE concentrate needed for the the FAE reagent to attain a count rate of 933,000 (866,000-1,000,000):

          933,000 RLU/25uL
----------------------- X Batch Volume (mL) X 0.8 = Volume of Concentrate RLU/25uL

Volume of Concentrate to add: ____________________

___ 8. Mix for 1 hour on a magnetic stirrer.

___ 9. Run totals on the FAE Reagent as in step 5 above and record below.

RLUs for 25uL FAE Reagent:
___________________________
___________________________
___________________________
___________________________

Mean RLUs for 25uL FAE reagent: ____________________

___ 10. If the RLUs/25uL are between 866,000 and 1,000,000 proceed with Step 14.

___ 11. If the RLUs/25uL are below the target range, calculate and record the additional volume of FAE concentrate to add.

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\frac{933,000 \text{ RLU}}{\text{Current RLU}} \times \frac{\text{Volume of FAE Concentrate Added}}{\text{Volume of FAE Concentrate To Add}} \times 0.8 = \text{Volume of Concentrate to Add}
\]

Additional volume of Concentrate to add: ____________________

___ 12. Repeat steps 8 and 9, recording RLUs below.

RLUs for 25uL FAE Reagent:
___________________________
___________________________
___________________________
___________________________

Mean RLUs for 25uL FAE reagent: ____________________

___ 13. Repeat steps 11 and 12 until RLUs fall within the desired range.

___ 14. Bottle FAE Reagent in appropriate size nalgene bottles. Label with FAE, date prepared, initials, etc. according to the Procedure for Labeling Secondary Reagent Containers. Store refrigerated.