Title: Procedural Worksheet for Preparation of FMP Reagent

Laboratorian: _______________________
Date of Preparation: _______________________
Lot of FMP Wetcake: _______________________
Concentration of Wetcake(mg/mL): _______________________
Lot Expiration Date: _______________________
Date of Buffer: _______________________
Batch volume: _______________________

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

_____ 1. Obtain required volume of filtered FMP Reagent Buffer from the A&R Core. (Eight liters is a convenient volume)

_____ 2. Obtain the Fisher Hematology/Chemistry Mixer Model 346, the large plastic carbuoy with spiget, and ring stand-motorized-stirer from room A122.

_____ 3. After suspending all PMP particles by swirling and inverting the flask, mix the wetcake (PMP concentrate) for at least one hour by taping it on the Fisher Mixer Model 346.

_____ 4. Calculate the volume of FMP wetcake required for the entire batch and record the information below.

\[
\text{Batch Size(mL)} \times 0.0675 \text{ mg/mL} = \text{Concentration of wetcake (mg/mL)} \times \text{(Volume Wetcake Required For Batch, mL)}
\]

_____ 5. Measure out the calculated volume of wetcake into a 50mL conical tube. Magnetically separate and wash the wetcake three times. Each wash will consist of resuspending the wetcake to its original volume with FMP Reagent Buffer, mixing for 5 minutes by inversion and magnetically separating for 5 minutes by attaching the conical tube to the bar-shaped magnet with a rubber band. After each wash and separation, carefully pour off the supernatent while the tube stays in contact with the magnet.

_____ 6. After the third wash, resuspend the wetcake to its original volume with FMP Reagent Buffer, mix for 5 minutes and add it to the balance of the buffer in the plastic carbuoy retaining about 100mL of buffer to further wash the PMP particles from the conical tube.
7. Mix the FMP thoroughly for one hour using the ring stand-motorized-stirrer.

8. Check the new batch of FMP by running a standard curve and controls in duplicate using either the uFSH or serum FSH protocol. RLU values for the standards should be approximately the same as previous assays and controls should be within acceptable ranges.

9. If the standard RLU values are low determine the amount of extra FMP wetcake to add.

\[
\text{Target RLU} \times \frac{\text{Volume of FMP}}{\text{Wetcake}} - \frac{\text{Volume of FMP}}{\text{Wetcake}} = \frac{\text{Volume of Wetcake}}{\text{To Add}}
\]

Additional volume of Wetcake to add: ____________

Repeat steps 5 – 8.

10. When the FMP Reagent is acceptable, bottle it in appropriate size nalgene bottles. Label with FMP, date prepared, initials etc. according to the Procedure for Labeling Secondary Reagent Containers. Store refrigerated.