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SPARC - Setting up the BEADS for the Millipore Metabolic Rat Milliplex Assay

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ABSTRACT

The beads are made for two half plates and are good for 1 month.

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GUIDELINES

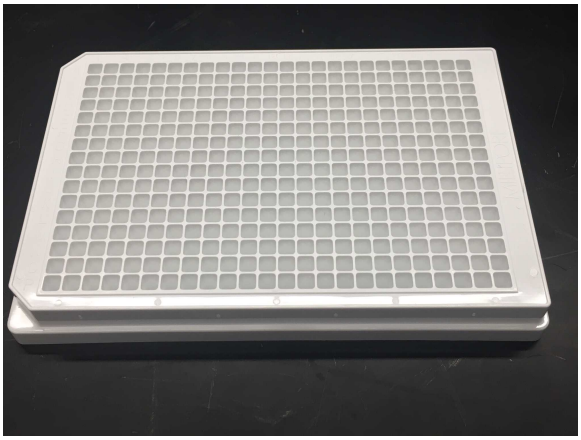
The beads are made for two half plates and are good for 1 month.

BEFORE STARTING

The following are required:

- Teal clip tips
- 5x16 Eppendorf rack
- Multi-vortex tube holder
- Vortex
- Large bucket (useful when dealing with wet tubes when vortexing)
- Sonicator (use distilled H_20) (in our lab use gallon jar with green label)
- Tray labeled for mixed beads
- Tray labeled for diluents

1 Before making the beads, the filter plate must be pre-wet for at least **00:10:00** .



2 Add **75 µL assay buffer** to each well using the teal (15-1250µl) Eclip tip using only 6 pipette tips (see below for diagram), using settings **Preset>stepper>pre-step, 75ul, 12x, 3, 4** (will pull up 900µl).

Use only 6 tips (pipette twice in the same row first in tube 1 and then in tube 2) to pipette like

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so:
Row
# # # # # # # # # # # #
1 1 1 1 1 1
2 2 2 2 2 2

The spacing should be set to narrowest and you will be adding to every other row. Do some mini drops on the plate to get the liquids to the bottom of the wells.

3 Replace plate lid and place plate on the blue shaker, making sure to tape it securely, before shaking the plate for at least **00:10:00** (longer is okay).

4 Take each bead vial out and verify the bead identity. **Pay attention to active vs total forms of analytes.**

5 Unscrew the lid a little and screw it back on (but not super tight).

6 Place each vial into the multi tube vortex holder.

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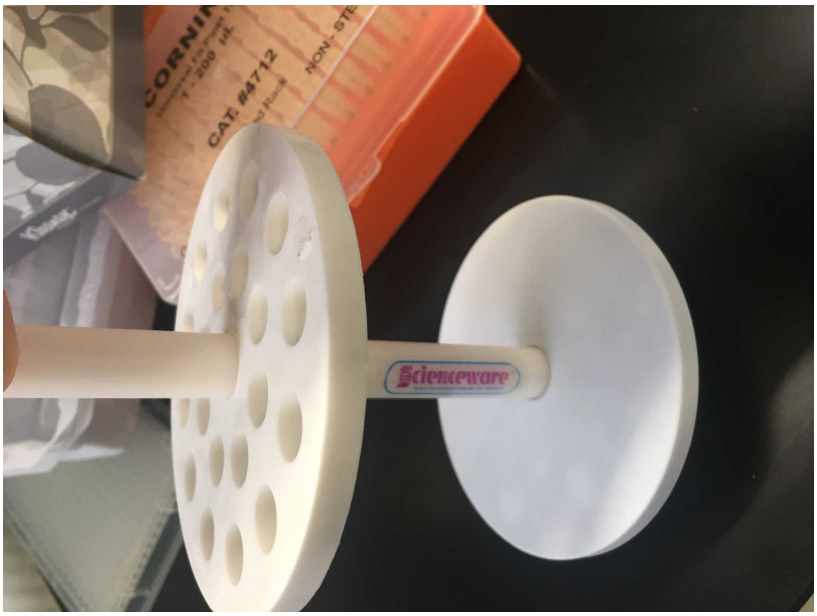

7 Place the vortexer in the large bucket (to prevent splashing of water on outside of vials). (if you don't have it in large bucket, water can be sprayed all over the lab bench!)

8 Label a blue screw-top 5mL tube with mixed beads and date, and wrap it in aluminum foil to **protect it from light**.

9 Gather tray labeled with "**bead diluents 384 well half plate**" and "**mixed beads tray**" and make sure they are clean and dust-free.

10 Fill "bead diluents 384 well half plate" with the whole vial of bead diluents.

11 Using clip-tip 15-1250µL teal electronic pipettor, select "program forward" with the options:
- Set volume = 100
- Speed up = 5
- Speed out = 2

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12 Set up tip box to have 4 rows of 5 tips across.

- * * * * *
- * * * * *
- * * * * *
- * * * * *

13 **Check Branson Sonicator.** The water must be at the water line level. If it is not, raise using distilled H_2O.

14 Place all 10 tubes in the multi-tube vortex holder and put the lid on.

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15 Sonicate samples for **00:00:30** .

> **Do not get the caps wet!**

16 Vortex the tubes for **00:01:00** .

> Work quickly when working with beads.

17 Take the bead vials out and put them in the Eppendorf rack, with 5 tubes on one side and 5 tubes on the other side.

18 Open all of the lids (leave them next to the vials)

19 Set the pipette to the widest setting (12.9).

20 Take up 100µl from the first 5 bead vial tubes (each tube contains 200µl beads).

21 Dispense into the mixed bead tray. **DO NOT eject tips.**

22 Take the remaining 100µl from the original 5 bead vial tubes and dispense into the mixed bead tray.

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23 Use new tips to take up 100µl bead diluents and rinse the tube with the 100µl bead diluents before dispensing into the mixed bead tray.

24 Rinse the tubes for the second time with 100µl bead diluents and dispense into the mixed bead tray.

25 Repeat with the second 5 tubes:

25.1 Take up 100µl from the second 5 tubes (each tube contains 200µl beads).

25.2 Dispense into the mixed bead tray. **DO NOT eject tips.**

25.3 Take the remaining 100µl from the original 5 bead vial tubes and dispense into the mixed bead tray.

25.4 Use new tips to take up 100µl bead diluents and rinse the tube with the 100µl bead diluents before dispensing into the mixed bead tray.

25.5 Rinse the tubes for the second time with 100µl bead diluents and dispense into the mixed bead tray.

26 Use a 1mL pipette to mix the beads very well and transfer to the 5mL tube with aluminum foil around.

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