5. Add 70 µL of SPRIselect and 90 µL of 100% Isopropanol (American Bioanalytical, A07015) to the supernatant containing wells.

6. Mix the total reaction volume by pipetting 10 times and incubate at room temperature for 5 minutes. Place the reaction vessel on the magnet plate or magnet stand and allow the SPRI beads to settle to the magnet for 10 minutes or until the supernatant turns completely clear.

7. Transfer the entire clear supernatant (approximately 300 µL) containing the majority of the miRNA into a new well. ***NOTE:*** *Care should be taken not to aspirate a significant amount of beads during this step as the majority of the intermediate RNA fragments are associated with the beads. Accidentally aspirating the beads into the supernatant will carry over RNA fragments between 100-200 nts to the next step*

8. Add 90 µL of SPRIselect, 270 µL of 100% Isopropanol into the supernatant containing wells.

9. Mix the total reaction volume by pipetting 10 times and incubate at room temperature for 5 minutes.

10. Place the reaction vessel on the magnet plate or magnet stand and allow the SPRI beads to settle to the magnet for 15 minutes or until the supernatant turns completely clear.

11. Remove and discard the clear supernatant. ***NOTE:*** *Care should be taken not to aspirate a significant amount of beads during this step as the desired miRNA is associated with the beads. Significant bead loss will result in reduced yield.*

12. With the reaction vessel still on the magnet, add 500 µL of 85% non-denatured ethanol (freshly prepared/ diluted from 100% ethanol, American Bioanalytical, part # AB00138) and incubate at room temperature for 30 seconds without disturbing the beads.

13. Remove and discard the ethanol supernatant. ***NOTE:*** *Care should be taken not to aspirate a significant amount of beads during this step as the desired miRNA is associated with the beads. Significant bead loss will result in reduced yield.*

14. Repeat the steps 12-13 for second ethanol wash.

15. Let the beads air dry for 5-10 minutes.

16. To elute the sample, remove the reaction vessel from the magnet plate or magnet stand, add 20-50 µL of molecular biology grade RNAase/DNase free water or 10 mM Tris-HCl(pH8.5). ***NOTE:*** *At least 20 µL of nuclease free water is required for the elution step. Make sure the beads are completely suspended to avoid yield loss.*

17. Mix the total elution volume by pipetting 10 times and incubate at room temperature for 2 minutes.

18. Place the reaction vessel on an appropriate magnetic plate and allow the SPRI beads to settle to the magnet for 2 minutes or until the supernatant turns completely clear.

19. Transfer the eluted miRNA sample to an appropriate storage vessel and keep at -80°C for long-term storage.

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