

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.



Beckman Coulter makes no warranties of any kind whatsoever expressed or implied, with respect to the method, including but not limited to warranties of fitness for a particular purpose or merchantability or that the method is non-infringing. All other warranties are expressly disclaimed. Use of the method is solely at your own risk, without recourse to Beckman Coulter.

© 2013 Beckman Coulter Life Sciences. All rights reserved.

Beckman Coulter, the stylized logo, SPRI, SPRIselect are registered trademarks of Beckman Coulter, Inc. All other trademarks are the property of their respective owners. Printed in USA..

For Beckman Coulter's worldwide office locations and phone numbers, please visit www.beckmancoulter.com/contact

B2013-14460 IB-18478A

www.beckmancoulter.com

© 2013 Beckman Coulter, Inc.

PRINTED IN U.S.A.