Modified protocol for Isolating RNA from Grape Stems

1. Ground sample with liquid nitrogen.

2. Weight 10-50 mg ground sample into a tube contains 400µl CPL Buffer. Mix the sample throughly by vortexing the tube 20 seconds.

3. (Optional) Incubate at 55-60C for 5 minutes.

4. Add 400 µl chloroform and mix throughly by vortexing for 20 seconds.

5. Centrifuge at ≥13,000 x g for 5 minutes.

6. Transfer 300µl of upper aqueous phase into a new 1.5 ml microtube.

7. Add equal volume of the RB Buffer followed by 325 µl absolute ethanol (96-100%). Mix the sample thoroughly by vortexing.

8. Load 600 µl of the sample into the HiBind RNA column. Centrifuge at 10,000 x g for 1 minutes. Discard the flow-through and re-use the collection tube.

9. Apply remaining sample to the HiBind RNA column by repeating step 8. Discard the flow-through and collection tube.

10. Place the RNA column into a new collection tube. Add 500 µl RNA Wash Buffer I into the column . Centrifuge at 10,000 x g for 1 minutes. Discard the flow-through and re-use the collection tube.

11. Add 500 µl RNA Wash Buffer II into the column . Centrifuge at 10,000 x g for 1 minutes. Discard the flow-through and re-use the collection tube.

12. Add 500 µl RNA Wash Buffer II into the column . Centrifuge at 10,000 x g for 1 minutes. Discard the flow-through and re-use the collection tube.

13. Centrifuge the empty column for 2 minutes to dry the column.

14. Place the column into a new 1.5 ml centrifuge tube. Add 100 µl DEPC Water into the center of the membrane in the column. Incubate at RT for 2 minutes.

15. Centrifuge at maximum speed (>13,000 x g) for 1 minutes to elute RNA.

Modified protocol for Isolating RNA from Grape Leaves

1. Ground sample with liquid nitrogen.

2. Weight 10-50 mg ground sample into a tube contains 400µl CPL Buffer. Mix the sample throughly by vortexing the tube 20 seconds.

3. Add 400 µl chloroform and mix throughly by vortexing for 20 seconds.

4. Centrifuge at ≥13,000 x g for 5 minutes.

5. Transfer 300µl of upper aqueous phase into a new 1.5 ml microtube. Add 300µl of isoproanol to precipitate nucleic acid.

6. Centrifuge at 13,000 x g for 5 minutes. Remove the supernatant and dissolve RNA by 300ul DEPC Water.

7. Add equal volume of the RB Buffer followed by 325 µl absolute ethanol (96-100%). Mix the sample thoroughly by vortexing.

8. Load 600 µl of the sample into the HiBind RNA column. Centrifuge at 10,000 x g for 1 minutes. Discard the flow-through and re-use the collection tube.

9. Apply remaining sample to the HiBind RNA column by repeating step 8. Discard the flow-through and collection tube.

10. Place the RNA column into a new collection tube. Add 500 µl RNA Wash Buffer I into the column . Centrifuge at 10,000 x g for 1 minutes. Discard the flow-through and re-use the collection tube.

11. Add 500 µl RNA Wash Buffer II into the column . Centrifuge at 10,000 x g for 1 minutes. Discard the flow-through and re-use the collection tube.

12. Add 500 µl RNA Wash Buffer II into the column . Centrifuge at 10,000 x g for 1 minutes. Discard the flow-through and re-use the collection tube.

13. Centrifuge the empty column for 2 minutes to dry the column.

14. Place the column into a new 1.5 ml centrifuge tube. Add 100 µl DEPC Water into the center of the membrane in the column. Incubate at RT for 2 minutes.

15. Centrifuge at maximum speed (>13,000 x g) for 1 minutes to elute RNA.