Most plant viruses have RNA genomes that can be single stranded or double stranded. During replication of viruses in plant, high molecular weight double-stranded ribonucleic acid (dsRNA) is produced as an intermediate of viral replication. Although "healthy plants" do not normally contain high molecular weight dsRNAs, some plant species and specific cultivars contain dsRNAs which are the genome of cryptic viruses or endornaviruses. Considering the economic damage and quarantine purpose, the detection and analysis of dsRNAs obtained from plants infected with RNA viruses has been used as an alternative tool for plant virus diagnosis. Since the size and number of dsRNAs from virus infected plants are unique for each virus species, cumulative evidence can be eventually built for identification of viral species.

Contrast to the complicated method of reverse transcription-polymerase chain reaction (RT-PCR) requires specific primers and expensive apparatus, monitoring the presence of dsRNA in the unidentified plant sample can be the first line of diagnosis especially in the resource limited countries. The monitoring can be also very effective in the case of bulk sample handling such as quarantine related process or plant cultivation business.

So far the method most commonly used for purification of these dsRNAs from plant tissues involves phenol extraction followed by adsorption to fibrous cellulose using ethanol. Analysis of the purified dsRNA is normally done by gel electrophoresis. It is rather labor some and lengthy steps that is not feasible for multiple samples. Also the handling or organic solvent is tedious and not pleasant process.

5 min dsRNA Extraction kit uses totally novel technology for purification of dsRNA. From any source of plant tissue, dsRNA can be specifically purified without any contamination of genomic DNA or single stranded RNA. Total dsRNA of plant can be purified literally in 5 minutes or less. One can do the monitoring of dsRNA from any plant tissue for more than 200 samples in a day. Because of the speed and efficacy, the Kit can serve the innovative tool in primary plant viral diagnosis without the wasting of resources and endeavor.
The kit also can be used for a simple purification of dsRNA from the mixture of ssRNA or DNA. dsRNA can be purified from the total mixture of nucleic acid from \textit{in vivo} synthesized dsRNA from HT115 strain or \textit{in vitro} transcriptional reaction. The final eluted samples are enriched with dsRNA without contamination of other RNA. The dsRNA is perfect materials for RNAi assay in insects, plants, and invertebrates such as shrimp.

- Catalog #; R-1003
- The fastest and the simplest but most reliable Nucleic Acid extraction system.
- Total procedure in 5 minutes.
- Purification of dsRNA from any plant tissue, transcriptional reaction, or total mixture of cellular Nucleic Acid
- Exclusive purification of dsRNA without any ssRNA or genomic DNA contamination.
- The dsRNA from virus or an artificial gene extraction.
- An ideal tool for primary diagnostic of viral infection in plants
- Perfect tool for dsRNA and shRNA expression or delivery studies
- dsRNA control (600 bp, 2 ug/ul and total 200 ul) is included.
- No liquid nitrogen or an expensive homogenizing device.
- No organic solvent in any of step.
- No damage to RNA quality during extraction procedure.
- Almost all dsRNA in the sample can be recovered.
- All procedure at ambient temperature without any cold or freezing step.
- Single column format.
- The kit can be stored at ambient temperature for more than a year.
- One kit for 50 dsRNA purification.
- Not appropriate for purification of small dsRNA such as siRNA or miRNA. Please use 5 min miRNA Extraction kit (R-1002) for miRNA or siRNA.
- The extracted RNA can be used in any downstream steps such as qRT-PCR reaction without any inhibitory effect.
Storage Conditions and Product Stability
All components should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

Precautions and Disclaimers
This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Customer-Supplied Reagents and Equipment
- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- Vortex
- Microcentrifuge tube pestle and small surgical scissors (not included in this kit). You may purchase it through Biofactories, Catalogue number ETC-1001.
- Please refer the visual instruction of the pestle and scissors on Youtube: [https://www.youtube.com/watch?v=a2GbEphe0iQ](https://www.youtube.com/watch?v=a2GbEphe0iQ)
- Microcentrifuge tube pestle and small surgical scissors
- 99-100 % isopropanol
- 96-100 % ethanol

Kit components for 1 box/50 prep

Microcentrifuge tube pestle and small surgical scissors are not included in this kit. You may purchase it through Biofactories web ([www.5minDNA.com](http://www.5minDNA.com) Biofactories catalogue number; ETC-1001).

<table>
<thead>
<tr>
<th>Component</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column/collection tube</td>
<td>50 ea.</td>
</tr>
<tr>
<td>dsRNA Solution I</td>
<td>30 ml</td>
</tr>
<tr>
<td>dsRNA Solution II</td>
<td>2 ml</td>
</tr>
<tr>
<td>RNA Washing Solution</td>
<td>18 ml</td>
</tr>
<tr>
<td>RNA Elution Buffer</td>
<td>7.5 ml</td>
</tr>
<tr>
<td>dsRNA control</td>
<td>200 ul (2ug/ul)</td>
</tr>
<tr>
<td>Product insert</td>
<td>1 ea.</td>
</tr>
</tbody>
</table>
5 min dsRNA Extraction Kit®

Before Starting

1. Video manual for the kit is available on Youtube:
   https://www.youtube.com/watch?v=50vL27LbR1I
   https://www.youtube.com/watch?v=zIOiKcXhwLo&t=4s
2. Add 25 ml of 99 % isopropanol to dsRNA Solution II. Mix well. Mark on the
   labels that isopropanol is added. Store it at room temperature.
3. Add 42 ml of 96-100% ethanol to RNA Washing Solution. Mix well. Mark on
   the labels that ethanol is added. Store it at room temperature.
4. Warp the distal end of microcentrifuge tube pestle with folded paper towel and
   type it. With this set up, you can apply more pressure to the pestle for more
   effective tissue extraction.
5. The method for the squeezing samples by the microcentrifuge tube pestle is
   linked to a video file in YouTube
   (https://www.youtube.com/watch?v=a2GbEphe0iQ).
6. The microcentrifuge tube pestle can be reused after wash in running tap water,
   spray with 70% ethanol, and wipe out with a clean paper towel.
Procedures for 5 min dsRNA extraction from plant

Important notice; all centrifugation in 13,500 rpm.

1. Add less than 200 mg of plant tissue to the 1.5 ml microtube (not supplied) using a pinset.
2. Twist squeeze the sample with microcentrifuge tube pestle to fine pieces. Twist squeeze is essential for more effective extraction and more yield of RNA.
3. Add 550 ul of dsRNA Solution I and wash out the pestle.
4. (optional) You may add 2 ul of control dsRNA to the sample to test the recovery of dsRNA by the column. By monitoring the recovery of 600 bp control RNA, you can make sure for the purification procedure.
5. Closed the cap and loosened the pellet by scraping the tube for 5-6 times over an uneven surface such as a microcentrifuge tube rack.
6. Vortex for 30 sec. and microcentrifuge for 1 min.
7. Transfer 450 ul of lysate to new microtube. (Notice; Carry over some debride in this step doesn’t interference the RNA extraction).
9. Load all solution to the supplied column and centrifuge for 15 sec.
10. Discard the flowthrough. Reassemble the spin column with its collection tube.
11. Apply 700 ul of RNA Washing Solution and centrifuge for 15 sec. Discard the flowthrough.
13. Replace the collection tube with a clean microcentrifuge tube (not supplied).
15. Close the cap and vortex for 15 sec.
Procedures for 5 min dsRNA purification from solution

Important notice;

- All centrifugation in 13,500 rpm.
- The mixture of total nucleic acid or in vitro transcription reaction can be used for further dsRNA purification.

1. Add 2 times volume of 100% ethanol to the solution contains dsRNA and spin for 1 min.
2. Discard all solution without disturbing any pellet.
3. Add 450 ul of dsRNA Solution I.
4. Closed the cap and loosened the pellet by scraping the tube for 5-6 times over an uneven surface such as a microcentrifuge tube rack.
5. Vortex for 10 sec.
7. Load all solution to the supplied column and centrifuge for 15 sec.
8. Discard the flowthrough. Reassemble the spin column with its collection tube.
10. Apply 400 ul of RNA Washing Solution and centrifuge for 30 sec. Discard the flowthrough.
11. Replace the collection tube with a clean microcentrifuge tube (not supplied).
12. Add 25-100 ul of RNA Elution Buffer to column.
13. Close the cap and vortex for 15 sec.
15. Reload the flow through and centrifuge for additional 30 sec.

**Example of test**

![Image of agarose gel](dsRNA.png)

dsRNA extraction from plant tissue using 5 min dsRNA Extraction Kit. Tomato leaves (0.1 g) were minced to fine pieces by microcentrifuge tube pestle and mixed final 5 ul of control dsRNA (length of 600 bp). RNA was extracted either by 5 min Tissue RNA Extraction Kit (lanes 1 and 2) or 5 min dsRNA Extraction Kit (Lanes 3 and 4) and eluted to final 100 ul. Ten ul of each sample was analyzed on 1 % agarose gel. Exclusive dsRNA can be purified by the 5 min dsRNA Extraction Kit.

![Image of agarose gel](dsRNA2.png)

dsRNA extraction from plant tissue using 5 min dsRNA Extraction Kit. Tomato leaves (0.1 g) were minced to a small pieces using microcentrifuge tube pestle and spiked with final 10 ug of mixture of three dsRNAs size of 1 kb, 0.5 kb, and 0.25 kb. Total Nucleic acid (lanes 2 and 3, prepared by 5 min Plant DNA/RNA Extraction kit) or dsRNA (lanes 4 and 5, by 5 min Plant dsRNA Extraction Kit) was purified based on the supplied protocol and analyzed on 1 % agarose gel. Lane 1 is the total input of each dsRNA spiked to the plant tissue lysate. dsRNA can be exclusively purified without any ssRNA or genomic DNA by 5 min dsRNA Extraction kit.
Efficient recovery of dsRNA by 5 min dsRNA Extraction Kit. Four artificial dsRNA with size of 1.5, 1, 0.5, and 0.25 Kb was mixed and used for the recovery efficiency test of the kit. Lanes 1 to 3; total input RNA before purification, lanes 4 to 6; the same amount of each input RNA after purification. Almost 100% of each dsRNA can be recovered after the purification by the kit.

dsRNA extraction from plant tissue using 5 min dsRNA Extraction Kit. Tomato leaves (0.1 g) were minced to fine pieces and RNA was extracted by 5 min dsRNA Extraction Kit and eluted in final 100 ul of elution buffer. Ten ul of each sample was analyzed on 1.5 % agarose gel. Lanes 1 and 2; RNA sample from plant that express an artificial 350bp dsRNA. Lane 3; sample from the plant without the dsRNA expression. Exclusive dsRNA can be purified by the 5 min dsRNA Extraction Kit even for endogenous dsRNA.

For diagnostic application of the kit for plant virus infection, the dsRNA can be extracted from the plant samples and determine the presence of target viral sequence using RT-qPCR. Two hundred mg of leaf sample of cowpea was used to test the infection by cowpea mosaic virus (CPMV) using appropriated primers. The sample was squeezed with mortar and pestle as described elsewhere (https://www.youtube.com/watch?v=a2GbEphe0iQ). The supernatant was harvested after 15 second spin and mixed with the same volume of isopropanol. The mixture was processed as described in the manual which takes less than 5 minutes. The final eluted sample was used for qRT-PCR.
## Quality of RNA from the kit

<table>
<thead>
<tr>
<th></th>
<th>200 mg</th>
<th>A260/A230</th>
<th>A260/A280</th>
<th>Conc.(ng/㎕)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowpea leaf</td>
<td>Infected with Cowpea mosaic virus (CPMV)</td>
<td>1.496</td>
<td>1.967</td>
<td>470.4</td>
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</table>

### RT-qPCR

<table>
<thead>
<tr>
<th></th>
<th>Infected Virus</th>
<th>NTC1</th>
<th>NTC2</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracted dsRNA</td>
<td>Cowpea mosaic virus (CPMV)</td>
<td>37.05</td>
<td>N/A</td>
<td>18.68</td>
<td>18.64</td>
<td>19.66</td>
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</tbody>
</table>
Technical Support

Biofactories’ Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of our products. If you have any questions or experience any difficulties regarding products, please do not hesitate to contact us. For technical assistance and more information, please contact our Technical Support Team through email at techsupport@5minDNA.com.