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ProductInformation

Spectrum™ Plant Total RNA Kit

Product Codes **STRN10, STRN50, STRN250**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description
Plants are well known for their diversity in secondary metabolites. Some plant tissues are enriched in polyphenolic compounds or tannins, and others are enriched in polysaccharides. These secondary metabolites often interfere with RNA isolation and its use in downstream applications. Laborious procedures as well as hazardous organic extractions are often required to prepare RNA from such plant tissues.

Sigma's Spectrum™ Plant Total RNA Kit employs a novel purification chemistry to overcome these interfering materials in a simple and streamlined manner, without using hazardous organic solvents such as phenol and chloroform. As a result, the Spectrum Plant Total RNA Kit provides a convenient method for purifying high quality total RNA from demanding plant species and tissues, such as spruce, pine, cotton, grape, maple, potato tuber, canola seed, and corn seed; as well as common research plant species, such as *Arabidopsis*, tomato, corn, rice, and soybean.

Plant tissues are ground to a fine powder in liquid nitrogen and lysed in a lysis solution that releases RNA and at the same time inactivates ribonucleases and interfering secondary metabolites, such as polyphenolic compounds. After the removal of cellular debris, RNA is captured onto a binding column using a unique binding solution, which effectively prevents polysaccharides as well as genomic DNA from clogging the column. Residual impurities and most residual genomic DNA are removed by wash solutions, and purified RNA is eluted in RNase-free water. Up to 100 µg of total RNA can be purified from 100 mg of plant material in 30 minutes after the tissue has been ground. Typical RNA yields are between 20 to 60 µg, depending on the tissue type and developmental stage. Root, stem, and starch storage organ generally will have lower RNA yields (10-20 µg). Purified RNA is ready for immediate use in RT-PCR, Northern blots, and other applications.

RNA samples prepared by the Spectrum Plant Total RNA Kit contain only trace amounts of genomic DNA. However, for some very sensitive applications, such as RT-PCR of rare messenger RNA, it may become necessary to remove the trace amounts of genomic DNA from RNA preparations. Sigma offers On-Column DNase I Digest Set for removing traces of DNA during RNA purification (see Appendix I).

It is worth noting that small RNA molecules, such as microRNA, siRNA, tRNA, 5S rRNA, are not efficiently recovered using the standard procedure. If desired, these small RNA molecules can be recovered by increasing the amount of binding solution, as described in Protocol A in the RNA binding step.

Reagents Provided	Product Code	10 Preps	50 Preps	250 Preps
Lysis Solution	L 8167	10 ml	50 ml	250 ml
2-Mercaptoethanol	M 3148	0.15 ml	0.9 ml	2 x 2 ml
Binding Solution	L 8042	10 ml	50 ml	250 ml
Wash Solution 1	W 1141	10 ml	50 ml	250 ml
Wash Solution 2 Concentrate	W 3261	2.5 ml	15 ml	75 ml
Elution Solution	E 8024	1.5 ml	10 ml	50 ml
Filtration Columns	C 6866	10 each	50 each	5 x 50 each
Binding Columns	C 6991	10 each	50 each	5 x 50 each
Collection Tubes, 2 ml	T 5449	4 x 10 each	4 x 50 each	4 x 250 each

Reagents and Equipment Required but not Provided

- Mortar and pestle
- Liquid nitrogen
- Dry ice
- 2 ml microcentrifuge tubes for weighing tissue
- Microcentrifuge (12,000 x g or higher)
- Heat block
- 100% Ethanol, Product Code 45,983-6
- On-Column DNase I Digest Set, Product Code DNASE 10 and DNASE 70 (optional)
- Amplication Grade DNase I, Product Code AMP-D1 (optional)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

Store at room temperature.

Notes in avoiding RNase contamination

RNases are ubiquitous and very stable enzymes and generally do not need cofactors for enzymatic activity. While endogenous RNases are denatured during the lysis step and removed subsequently during RNA purification, care must be taken to avoid introducing exogenous RNases during RNA preparation, especially during the final wash and elution steps. The work area and the pipette set must be free of RNases, such as those often used in plasmid isolation. Use RNase-free pipette tips, preferably those with an aerosol barrier. The surface of the skin and the dust in the air can also be sources of RNase contamination. Always wear gloves and change them frequently. Keep bottles and tubes closed when not in use. Additional information on avoiding RNase contamination and working with RNA can be found in the References listed at the end of this technical bulletin.

Preparation Instructions

1. **Prepare Wash Solution 2**

   Wash Solution 2 is supplied as a concentrate. Prior to first time use, add an appropriate amount of 100% ethanol to the bottle of Wash Solution 2 Concentrate according to the table below. Mix briefly and store the diluted Wash Solution 2 tightly capped to prevent the evaporation of ethanol.

Product Code	Kit Size	Amount of Ethanol to Add
STRN10	10-prep	10 ml
STRN50	50-prep	60 ml
STRN250	250-prep	300 ml

2. **Prepare Plant Tissue Sample**

   Grind Plant Tissue

   Harvest plant tissue and submerge it in liquid nitrogen as soon as possible to prevent RNA degradation. Grind the tissue to a fine powder in liquid nitrogen using a mortar and pestle. RNA yield is often dependent upon how fine the plant tissue has been ground, especially if the tissue is difficult to grind. For best practice, place the mortar on dry ice and keep the plant material frozen at all times.

   Note: Fresh or frozen tissue can be used as starting material. Ground plant material can be stored at –70 °C for several months before RNA purification. However, do not allow the frozen material to thaw before lysis solution is added.

   Weigh Tissue Sample

   After liquid nitrogen has evaporated from the frozen tissue powder, quickly weigh approximately 100 mg (90-110 mg) of the tissue powder in a 2-ml microcentrifuge tube (not supplied), pre-chilled on dry ice or in liquid nitrogen. Keep the weighed sample on dry ice or at –70 °C before lysis solution is added. ***Do not exceed 110 mg of tissue powder per tube, especially with difficult plant tissues, such as pine needles.***

   Note: For extremely difficult plant tissues, such as citrus leaves and old Red Maple leaves that may contain oily and/or gummy materials, ***start with 50 mg of plant tissue powder per preparation.***

3. **Prepare Lysis Solution/2-ME Mixture**

   Lysis Solution must be supplemented with 2-mercaptoethanol (2-ME) before use. Transfer a pre-determined amount of Lysis Solution to a clean conical tube and add 10 µl of 2-ME for every 1 ml of Lysis Solution and mix briefly. Each RNA preparation requires 500 µl of the mixture. Some extra solution will be needed for pipetting allowance when working with multiple samples. For best results, prepare the mixture as close to the time of use as possible. Use of a Lysis Solution/2-ME mixture more than one day old after preparation may result in reduction in RNA yield.

4. **Assemble Column and Collection Tube**

   Insert a Filtration Column (blue retainer ring) into a 2-ml Collection Tube (provided) and close the lid for use in Step 2 under the Procedure. Likewise, insert a Binding Column (red retainer ring) into a 2-ml Collection Tube (provided) and close the lid for use in Step 3 under the Procedure.

Procedure

All centrifugation steps are performed at room temperature and at maximum speed (14,000-16,000 x g) in a standard microcentrifuge.

1. **Lyse Tissue Sample**
   Pipet 500 µl of the Lysis Solution/2-ME Mixture (see Preparation Instructions Step 3) to 100 mg of tissue powder and vortex immediately and vigorously for at least 30 seconds. Incubate the sample at 56 °C for 3-5 minutes. However, if the starting plant material is a starch storage organ, such as corn seed or potato tuber, incubate the sample at room temperature.

Note: Reduce the amount of tissue powder to 50 mg for extremely difficult plant tissues, such as citrus leaves and old Red Maple leaves (see **Weigh Tissue Sample**). Do not vortex the sample during or after the heat incubation.

2. **Pellet Cellular Debris**
   Centrifuge the sample at maximum speed for 3 minutes to pellet cellular debris.

Note: Mark the tube orientation before centrifugation so that the cellular debris pellet can be easily avoided in the next step. Some plant tissues, such as lipid storage tissues, may produce a viscous lysate and may require longer centrifugation times for pelleting the cellular debris.

3. **Filter Lysate**
   Pipet the lysate supernatant into a Filtration column (blue retainer ring) seated in a 2-ml Collection Tube by positioning the pipette tip at the bottom of the tube but away from the pellet. If there is a layer of floating particulates, position the pipette tip below the floating layer and away from the pellet before pipetting the supernatant. Do not be concerned with carry-over of the floating particulates to the Filtration Column, but avoid taking the pellet. Close the cap and centrifuge at maximum speed for 1 minute to remove residual debris. Save the clarified flow-through lysate.

Note: Some plant tissues may require longer centrifugation times for filtering the lysate. If all of the liquid has not passed through the Filtration Column after 1 minute of centrifugation, re-centrifuge the column for 3-5 minutes.

4. **Bind RNA to Column**
   Because plant tissues can vary greatly in water content as well as solute content, two protocols are recommended for different types of tissues:

   Protocol A is recommended for root, stem, flower, fruit, immature seed, leaf with high water content, or any succulent tissues that have a high water content and usually produce a very dilute cellular extract. Also use **Protocol A** for tissues that contain low levels of RNA, or to recover more of the small-sized RNA molecules (such as microRNA, tRNA, or 5S rRNA).

   Protocol B is recommended for conifer needle, leaf with normal water content (such as grape and tomato leaves), and starch storage organs.

Note: If you are not certain about the nature of your plant tissues, use **Protocol A**. Also use **Protocol A** for extremely difficult tissues, such as citrus leaves. You may also want to compare the two protocols with your tissues.

Protocol A

Add Binding Solution

Pipet 500 µl of Binding Solution into the clarified lysate and mix immediately and thoroughly by pipetting at least 5 times or vortex briefly. **Do not centrifuge**.

Note: If RNA level is expected to be low, or to recover more of the small-sized RNA, increase the amount of Binding Solution to 750 µl. Some extremely difficult tissues, such as citrus leaves, also require 750 µl of Binding Solution for optimal results.

Bind RNA

Pipet 700 µl of the mixture into a Binding Column red retainer ring) seated in a 2-ml Collection Tube. Close the cap and centrifuge at maximum speed for 1 minute to bind RNA. Decant the flow-through liquid and tap the Collection Tube (upside down) briefly on a clean absorbent paper to drain the residual liquid. Return the column to the Collection Tube and pipet the remaining mixture to the column and repeat the centrifugation and decanting steps.

Optional: For on-column DNase digestion, continue with the On-Column DNase Digestion procedure in Appendix I after the binding step.

Protocol B

Add Binding Solution

Pipet 250 µl of Binding Solution to the clarified lysate and mix immediately and thoroughly by pipetting at least 5 times or vortex briefly. **Do not centrifuge.**

Bind RNA

Pipet the mixture into a Binding Column (red retainer ring) seated in a 2-ml Collection Tube. Close the cap and centrifuge at maximum speed for 1 minute to bind RNA. Decant the flow-through liquid and tap the Collection Tube (upside down) briefly on a clean absorbent paper to drain the residual liquid. Return the column to the Collection Tube.

Optional: For on-column DNase digestion, continue with the On-Column DNase Digestion procedure in Appendix I after the binding step.

5. **First Column Wash**
   Pipet 500 µl of Wash Solution 1 into the column. Close the cap and centrifuge at maximum speed for 1 minute. Decant the flow-through liquid and tap the Collection Tube (upside down) briefly on a clean absorbent paper to drain the residual liquid. Return the column to the Collection Tube.

6. **Second Column Wash**
   Ensure that Wash Solution 2 Concentrate has been diluted with ethanol as described in the Preparation Instructions. Pipet 500 µl of the diluted Wash Solution 2 into the column. Close the cap and centrifuge at maximum speed for 30 seconds. Discard the flow-through liquid and tap the Collection Tube (upside down) briefly on a clean absorbent paper to drain the residual liquid. Return the column to the Collection Tube.

7. **Third Column Wash**
   Pipet another 500 µl of the diluted Wash Solution 2 into the column, close the cap and centrifuge at maximum speed for 30 seconds. Discard the flow-through liquid and tap the Collection Tube (upside down) briefly on a clean absorbent paper to drain the residual liquid. Return the column to the Collection Tube.

8. **Dry Column**
   Centrifuge the column at maximum speed for 1 minute to dry. Carefully remove the column-tube assembly from the centrifuge to avoid splashing the residue flow-through liquid to the dried column. If the residual flow-through liquid does accidentally contact the dried column, re-centrifuge the column for 30 seconds before proceeding to the elution step.

9. **First Elution**
   Transfer the column to a new 2-ml Collection Tube (provided). Pipet 50 µl of Elution Solution directly onto the center of the filter inside the column. Close the cap and let the tube sit for 1 minute. Centrifuge at maximum speed for 1 minute to elute. Purified RNA is now in the flow-through eluate and ready for immediate use or storage at –20 °C (short term) or –70 °C (long term).

10. **Second Elution (Optional)**
    If the expected RNA yield is > 20 µg, an additional 10-30% of RNA yield may be recovered from the column with a second elution. Transfer the column to a new 2-ml Collection Tube (provided). Pipet 50 µl of Elution Solution directly onto the center of the filter inside the column. Centrifuge at maximum speed for 1 minute to elute. Purified RNA is now in the flow-through eluate and ready for immediate use or storage at –20 °C (short term) or –70 °C (long term).

Results

Analysis of RNA

The concentration and quality of total RNA prepared can be determined by spectrophotometric analysis and agarose gel electrophoresis. Dilute the RNA (10 to 50-fold according to the expected yield) in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) and measure the absorbance at 260, 280, and 320 nm. Be sure to calibrate the spectrophotometer with the same solution. For best results, absorbance readings should be between 0.1 and 1.0 absorbance units (or within the linear range of the spectrophotometer). An absorbance of 1.0 at 260 nm corresponds to approximately 40 µg/ml of RNA. For most plant tissues, the ratio of absorbance at 260 to 280 nm, calculated by $(A_{260}-A_{320})/(A_{280}-A_{320})$, should be between 1.8 and 2.2. RNA integrity can be evaluated by agarose gel electrophoresis, analysis on an Agilent Bioanalyzer, or by capillary electrophoresis. The cytosolic 25 S and 18 S ribosomal RNAs should appear as discrete bands or peaks and in approximately 2:1 ratio. In addition, there might be some minor bands or peaks of chloroplast and mitochondria ribosomal RNAs.

Expected Yield

The yield of total RNA varies with tissue types, growth conditions, and developmental stages, as well as how fine the tissue has been ground. In general, younger and more rapidly growing plants or tissues will contain more RNA; and root, stem, and starch storage organ will contain less RNA. For example, in processing 100 mg of tissue sample, the Spectrum Plant Total RNA Kit has yielded 20-50 µg of total RNA from spruce and pine needles, 40-100 µg from grape and

tomato leaf tissues, and 10-20 µg from soybean root, corn root and corn seed.

RT-PCR

While most DNA is eliminated during total RNA purification with the Spectrum Plant Total RNA Kit, no single procedure removes 100% of the DNA. Therefore we recommend on-column DNase digestion (as described in Appendix I) for removing trace amounts of DNA during preparation of RNA to be used in RT-PCR if the PCR primers do not span an intron, or if pseudogenes that lack the intron may be present in the target tissue. Alternatively, the eluted RNA preparation can be digested with Amplification Grade DNase I (Product Code AMP-D1) before RT-PCR.

Appendix I

On-Column DNase Digestion

The Spectrum™ Plant Total RNA Kit efficiently removes most of the DNA during RNA purification. However, for very sensitive applications, such as quantitative RT-PCR, complete removal of traces of DNA may be necessary. Sigma offers an On-Column DNase I Digest Set (Product Codes DNASE 10 and DNASE 70) for removing trace amounts of DNA during RNA purification. After RNA has been bound to the Binding Column as described in Step 4 (**Bind RNA)** under the procedure, continue with the following steps to perform on-column DNase digestion.

1. Pipet 300 µl of Wash Solution I into the Binding Column, close the cap and centrifuge at maximum speed for 1 minute. Decant the flow-through liquid and tap the Collection Tube briefly on a clean absorbent paper to drain the residual liquid. Return the column to the Collection Tube.

2. For each digestion, combine 10 µl of DNase I (Product Code D 2816) with 70 µl of DNase digestion buffer (Product Code D 1566), and mix gently by pipetting. Do not vortex the DNase I vial or the mixture of DNase I and DNase I digestion buffer because DNase I is sensitive to physical denaturation.

3. Pipet 80 µl of the mixture directly onto the center of the filter inside the Binding Column. Close the cap and incubate the sample at room temperature for 15 minutes.

4. Pipet 500 µl of Wash Solution 1 into the Binding Column. Close the cap and centrifuge at maximum speed for 1 minute to remove the digested DNA. Decant the flow-through liquid and tap the Collection Tube briefly on a clean absorbent paper to drain the residual liquid.

5. Continue with the **Second Column Wash** step as described in Step 6 under the procedure.

Troubleshooting Guide

Problem	Cause	Solution
Filtration Column clogged	Pellet was transferred to the Filtration Column.	Avoid pipetting cellular debris pellet into the Filtration Column. If the pellet is too loose, repeat centrifugation for 3-5 minutes. If all of the liquid has not passed through the Filtration Column after 1 minute of centrifugation, re-centrifuge the column for 3-5 minutes.
	Sample was not heated at 56 ^{o}C during lysis.	Heat the plant material in lysis solution at 56 ^{o}C for 3-5 minutes. Do not vortex the sample during or after the heat incubation.
	Starchy sample was heated at 56 ^{o}C.	Incubate starch storage plant material (such as potato tuber, corn seed) in lysis solution at room temperature instead of at 56 ^{o}C.
	Plant material contains high level of lipids.	Increase the centrifugation time to 5 minutes to pellet the cellular debris and avoid taking the floating layers of lipid material.
Binding Column clogged	The mixture of clarified lysate and binding solution was too viscous.	Re-centrifuge the column for 2-3 minutes. If clogging persists, use a pipette tip to remove the trapped liquid before proceeding to the wash step. Reduce the starting plant material to 50 mg and increase the Binding Solution to 750 µl.

Low RNA yield	Too much tissue powder was used.	Do not exceed 110 mg of tissue powder. For extremely difficult tissues, such as citrus leaf and old Red Maple leaves, start with 50 mg of plant tissue powder. RNA yield will be greatly reduced or no RNA will be recovered if more than 110 mg of difficult plant material or more than 50 mg of extremely difficult plant material is used.
	Plant tissue was insufficiently ground.	Grind plant tissue to a fine powder in liquid nitrogen. RNA yields are highly dependent on how well the tissue was ground prior to isolation.
	Amount of Binding Solution was incorrect.	Use 500 or 750 µl of Binding Solution for root, stem, flower, fruit, or any succulent tissues that have a high water content and usually produce a very dilute cellular extract. Use 750 µl of Binding Solution for recovery of small amount of RNA or small-sized RNA molecules.
	Elution Solution was inappropriately dispensed into the column.	Pipet Elution Solution directly onto the center of the filter surface inside the Binding Column. Let the solution absorb into the filter for 1-2 minutes before centrifugation.
	Plant material contains small amounts of RNA.	Older root, stem, and tuber generally contain a small amount of total RNA. To increase the total RNA yield for each preparation, double the amount of starting material, Lysis Solution and Binding Solution.
	Improper storage of plant material	Flash-freeze any fresh plant material before storage at –70 °C. Do not let frozen plant material thaw before lysis solution is added.
	Lysis Solution/2-ME mixture was old.	Prepare a fresh solution. Do not use a Lysis Solution/2-ME mixture that is more than one day old.
RNA degraded	Plant tissues not stabilized properly	Submerge tissue in liquid nitrogen as soon as it is harvested and store on dry ice or at –70 °C to prevent RNA degradation.
	Contamination with exogenous RNases during or after purification	Avoid using a work area or a pipette set that has been used for plasmid isolation. Thoroughly clean any contaminated pipettes with RNaseZAP® (Product Code R 2020). Always wear gloves and keep bottles and tubes closed when not in use.
Residual genomic DNA	Too much tissue powder was used.	Do not exceed 110 mg of tissue powder. Residual amounts of genomic DNA will increase if too much tissue is used.
	No DNase treatment	In some tissues, residual genomic DNA may be detectable by RT-PCR. Perform on-column DNase digestion during RNA purification or treat the purified RNA sample with RNase-free DNase I (Product Code AMP-D1) after purification.
Inhibition in downstream experiments	Carry-over of salt or alcohol	Decant the binding and wash flow-through liquids and tap the Collection Tube (upside down) on an absorbent paper briefly to drain the residual liquid. After the column-drying step, if the residual flow-through liquid does accidentally contact the dried column, re-centrifuge the column again for 30 seconds before proceeding to the elution step.
	Failed to change the Collection Tube after the drying step.	Transfer the binding column to a new Collection Tube (provided) after the drying step and before eluting RNA.

References

1. Ausubel, F.M. et al., Current Protocols in Molecular Biology, John Wiley & Sons, NY, sections 4.1-4.10 (1995).

2. Farrell, Robert E., Jr., RNA Methodologies, 2nd Edition, Academic Press, NY, pp. 37-53 (1998). (Product No. Z35, 035-4)

3. Sambrook, J. et al. Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Plainview, NY, pp. 7.3-7.5 (1989).

FC/PHC 04/05-1

Related Products	Product Code
RNaseZAP	R 2020
Ethanol, Molecular Biology Grade	45, 983-6
MOPS-EDTA-Sodium Acetate Buffer	M 5755
RNA Sample Loading Buffers	R 1386 R 4268
RNA Markers, 0.2-10 kb	R 7020
On-Column DNase I Digestion Set	DNASE 10 DNASE 70
Deoxyribonuclease I, Amplification Grade	AMP-D1
Taq DNA Polymerase	D 1806
Deoxynucleotide (dNTP) Mix	D 7295
Enhanced Avian Reverse Transcriptase	A 4464
PerfectHyb™ Plus Hybridization Buffer	H 7033
Precast Agarose Gels, 1.25%, 8 well	P 6222

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