Full Spectrum™ Complete Transcriptome RNA Amplification Kit

Cat. # RA101A-1

User Manual

Store kit at -20°C on receipt

(ver. 6-061109)

Contents

I. Introduction and Background

- A. Overview 2
- B. RNA Amplification 2
- C. Overview of Protocol 6
- D. List of Components 7
- E. Additional Required Materials 7
- F. Procedural Guidelines 7

II. Protocol

- A. Starting RNA 8
- B. First-Strand cDNA Synthesis 9
- C. Second-Strand cDNA Synthesis and Amplification 10
- D. Gene-Specific Amplification Using Amplified cDNA 11

III. Troubleshooting

- A. No Product from cDNA Amplification 12
- B. No Product with Gene-Specific Primers 12

IV. Appendix

- A. Related Products 13
- B. Technical Support 13

V. Licensing and Warranty Statement 14

I. Introduction and Background

A. Overview

This manual provides details and information necessary to use the Full Spectrum™ Complete Transcriptome RNA Amplification Kit (*previously known as the Full Spectrum™ RNA Amplification Kit for degraded and non-degraded RNA*) to uniformly and reproducibly amplify limited amounts of non-degraded as well as degraded RNA to provide sufficient template for quantitative PCR of selected genes. To ensure optimal results, please read the entire manual before using the reagents and material supplied with this kit.

B. RNA Amplification

Quantitative Reverse Transcriptase PCR (qRT-PCR) is a widely used technique to determine mRNA levels of various genes. Although starting amounts for RT-PCR are relatively low—in the 0.1 to 1 μg range—it can be difficult to obtain sufficient amounts of starting material in cases where there is interest in looking at expression levels for a collection of transcripts. This problem is compounded since there is an increasing trend to use smaller, more well-defined samples, such as microdissected cells, tissue biopsies, fine needle aspirants, and other clinical samples. Most clinical tissue samples are preserved using formalin fixation followed by embedding in paraffin. RNA integrity in these formalin-fixed, paraffin-embedded (FFPE) tissues is typically very poor. As a result, it is very difficult to extract sufficient intact RNA for analysis. For these reasons, there is a strong need to have a reliable and robust approach to extract and prepare for expression analysis the small amount of RNA from non-degraded as well as degraded samples. The Full Spectrum™ Complete Transcriptome RNA Amplification Kit was developed to meet this need.

The Full Spectrum™ Complete Transcriptome RNA Amplification Kit provides an inexpensive method to amplify reverse transcribed RNA in a sequence independent, unbiased, and uniform manner. This approach maintains the relative levels of each transcript in the starting mRNA samples (Fig. 1)—even when using starting amounts of RNA as low as 5 ng (Fig. 2). In addition, this approach is faster, requires fewer steps, and is more convenient than other techniques, including T7-based amplification, SMART cDNA Amplification, or Ribo-SPIA amplification. Full Spectrum™ RNA Amplification offers the following benefits:

(1) High fidelity maintenance of relative transcription levels for each species of RNA in the starting mRNA population (Fig. 1)

(2) Uniform amplification across the whole transcript, generating overlapping cDNA fragments encompassing the starting mRNAs with no bias toward the 3'- or 5'-ends (Fig. 3)

(3) Maintain complete mRNA sequence even from degraded RNA

(4) Excellent results using degraded RNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples

(5) A true one-tube approach that does not require purification and provides you with amplified template in less than 3 hours (Fig. 4 in section I.C)

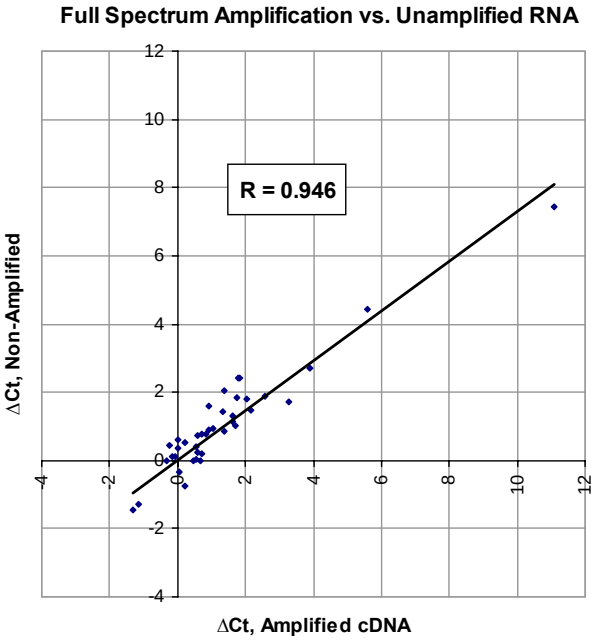

Fig. 1. High Amplification Fidelity. Using real-time Q-PCR, expression levels of 38 different genes from human kidney RNA were compared with their levels in a universal RNA control containing pooled RNA from 10 different tissues. One set of data was generated with unamplified RNA. The other set of data was generated with cDNA generated from the Full Spectrum™ Complete Transcriptome RNA Amplification Kit. A single real-time PCR reaction was performed on each sample for each gene primer set (replicate reactions were not done). For each data set, the Ct values for each gene were subtracted to provide the difference in expression levels between the two samples for each transcript. With the exception of a few genes that show very low difference in Ct (genes that are almost the same in both samples, the relative difference in expression levels was consistent between the unamplified and Full Spectrum-amplified RNA samples.

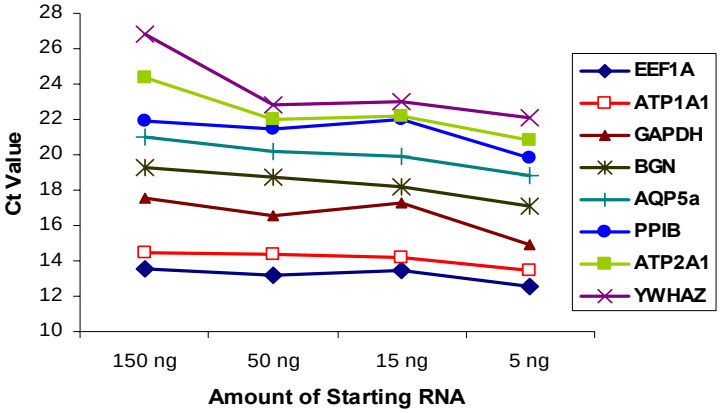

Fig. 2. Quantitative Amplification Regardless of Starting RNA Amount. The Full Spectrum™ approach was used to amplify 150 ng, 50 ng, 15 ng, and 5 ng of total RNA. Following the guidelines in step C.2 of the protocol, the number of amplification cycles was varied for each quantity of RNA to produce similar post-amplification yields. Following amplification, the expression levels of 8 genes in the four samples were measured by calculating the Ct values using qRT-PCR.

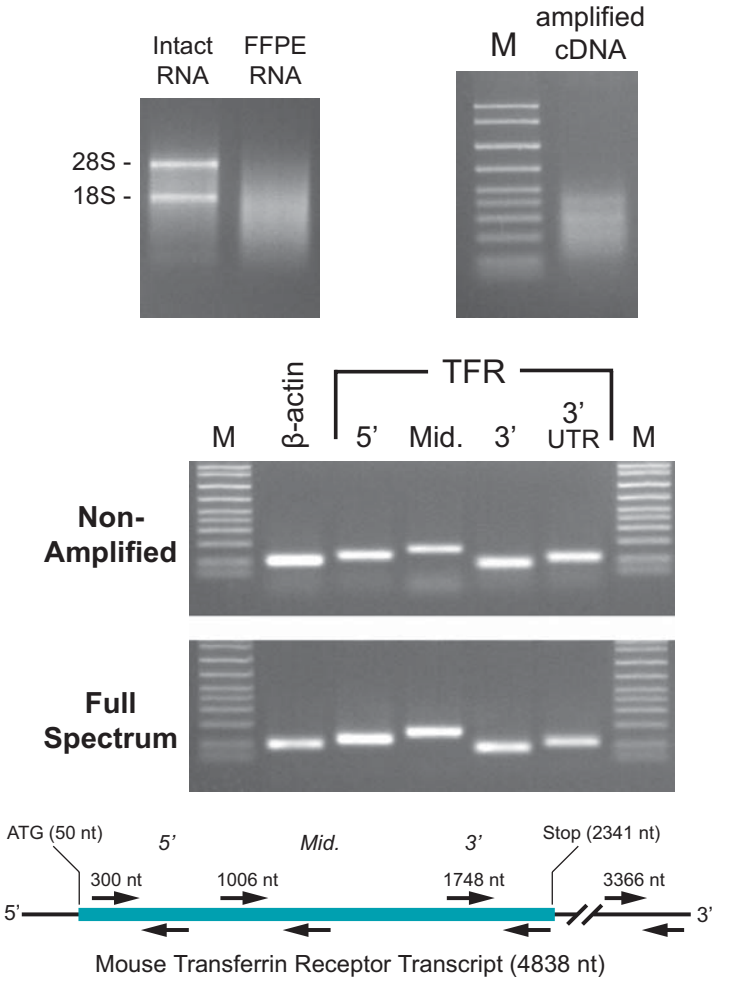

Fig. 3. mRNA Amplification from FFPE Tissue. To test the ability of the Full Spectrum™ Complete Transcriptome RNA Amplification Kit to amplify RNA derived from FFPE tissues, we isolated RNA from two 20 μm sections of mouse liver. As can be seen in the gel on the top left, the RNA from this tissue is substantially degraded. Following amplification, we ran samples of the cDNA product on a gel. As can be seen in the gel on the top right, the results show a range of cDNA products which are all relatively small in size. From the amplified material, the abundant β-actin transcript was amplified (as a positive control) and the transferrin receptor (TFR) transcript was amplified with primers targeted to the 5'-end, middle region, 3'-end, and 3'-UTR region. A diagram of the positions of the transferrin receptor primers is shown below the gels. The results of this analysis support the conclusion that all regions of the transcripts are amplified relatively equally using the Full Spectrum™ RNA Amplification Kit. **M:** 100 bp DNA Ladder, 50 bp – 2,000 bp

C. Overview of Protocol

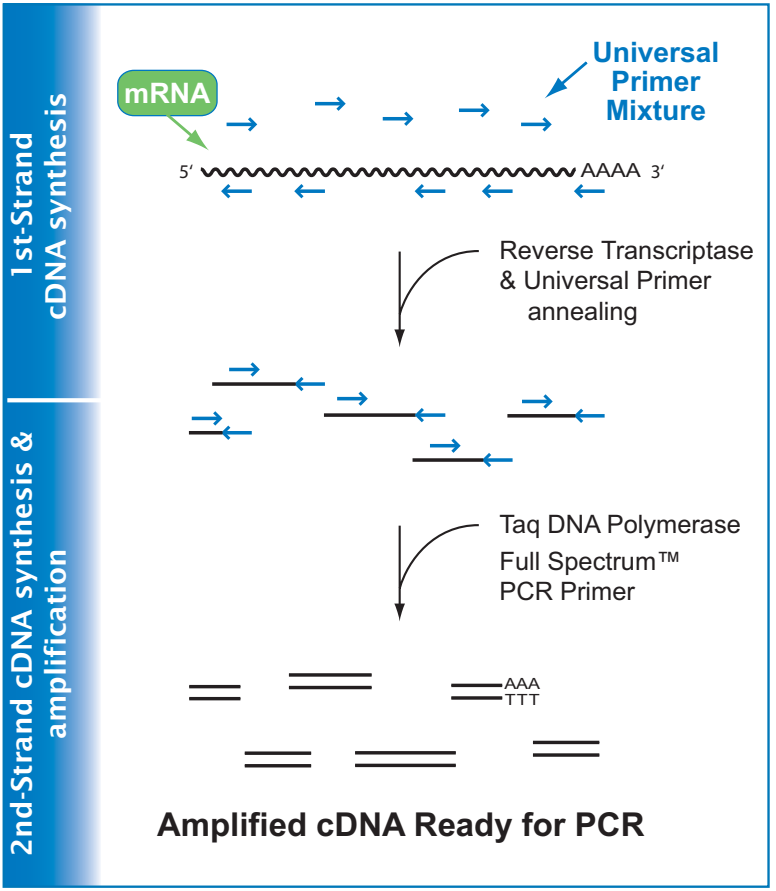

Fig. 4. Full Spectrum™ RNA Amplification Procedure

As can be seen in the flowchart outlining the procedure, RNA amplification with the Full Spectrum™ Kit involves just a couple of steps and less than 3 hours.

D. List of Components

Each Full Spectrum™ Complete Transcriptome RNA Amplification Kit provides enough material to amplify 10 RNA samples.

40	µl	Universal Primer Mixture
10	µl	Reverse Transcriptase
15	µl	Control RNA (25 ng/µl)
30	µl	5X Reverse Transcriptase Buffer
20	µl	Dithiothreitol (DTT)
50	µl	dNTP Mix
1.2	ml	RNase-free Water
150	µl	10X PCR Buffer
25	µl	Full Spectrum PCR Primer
25	µl	PCR Polymerase

The kits are shipped in blue ice and should be stored at -20°C upon receipt. Properly stored kits are stable for 1 year from the date received.

E. Additional Required Materials

- Thermocycler (with heated lid)
- 3% Agarose Gel in Tris-Borate EDTA (TBE) or Tris-Acetate EDTA (TAE) Buffer
- DNA Size Ladder with markers from 50 to 2,000 bp (Bio-Rad AmpliSize™ DNA Ladder; Cat. # 170-8200)
- Optional for samples from sources with high RNase activity: Ribonuclease Inhibitor (Ambion SUPERase-IN™; Cat. # 2694)

F. Procedural Guidelines

- Before dispensing, completely thaw all reagents. Vortex, to mix thoroughly, all reagents except for the enzymes. After adding reagents to the mixture, pipette up and down 5-10 times to ensure mixing.
- Briefly centrifuge each mixture once all the components have been added to ensure there are no reagents left on the sides of the tube, separated from the reaction mixture.
- When setting up multiple reactions, we recommend you prepare a master mix.
- Both first- and second-strand cDNA is relatively stable and can be stored for a few hours at room temperature or 4°C. For longer storage, place at -20°C.
- It is important to perform the amplification with the Control RNA provided with this kit. Without this control reaction, it will be difficult to troubleshoot any unexpected results.

II. Protocol

A. Starting RNA

1. Starting Amount of RNA.

   We recommend starting with approximately 100 ng of total RNA. Our studies have consistently shown greater than 95% representation of RNA species in the amplified population when compared with unamplified RNA with starting concentrations greater than 100 ng.

   The minimum starting concentration we recommend is 5 ng of total RNA. Typically, we obtain approximately 85% representation when starting with 5 ng.

2. Quality of RNA

   If possible, we recommend confirming the quality of your RNA before starting the amplification. The Agilent BioAnalyzer offers a convenient, sensitive, and reliable method to test small amounts of RNA.

   The Full Spectrum™ kit will generate representative amplified product with degraded RNA (see Fig. 5)

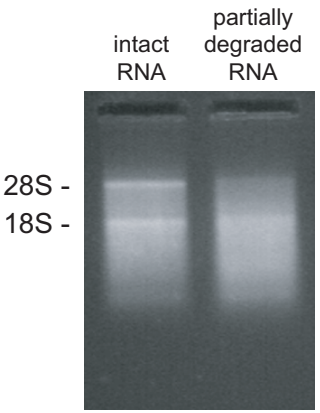

Fig. 5. Intact and Degraded RNA. The gel shows intact total RNA and total RNA that has been degraded. Both of these samples produced good results using the Full Spectrum™ RNA Amplification Kit (see Fig. 6).

B. First-Strand cDNA Synthesis

1. For each RNA sample, add the components to a 0.2 or 0.5 ml PCR tube in the order specified:

1.0 µl	RNase-free Water
2.0 µl	Universal Primer Mixture
2.0 µl	Total RNA
5.0 µl	Total volume

   Note: Because reagent volumes are small, accurate pipetting is critical. It is particularly important to have the correct amount of Universal Primer for the volume of the reaction.

2. Incubate the reactions at 70°C for 4 minutes, and then place the reactions at 25°C for 5 minutes.

3. While the reactions are incubating, set up a 2X Master Mix sufficient for the number of first-strand synthesis reactions you are processing. This is done by adding to a microfuge tube the volume of each of the following components multiplied by the number of reactions you are processing:

1.0 µl	RNase-free Water
2.0 µl	5X Reverse Transcriptase Buffer
1.0 µl	dNTP Mix
0.5 µl	Dithiothreitol (DTT)
0.5 µl	Reverse Transcriptase
5.0 µl	Total volume

   Note: If you have 2 reactions, you should have 10 µl of Master Mix; if you have 3 reactions, 15 µl, etc.

4. After incubating each of the first-strand reactions from step 2 for 5 minutes at 25°C, add 5 µl of the Master Mix set up in step 3.

5. Incubate the first-strand reactions for 50 minutes at 42°C, and then place them at 95°C for 5 minutes. The first-strand cDNA can be stored at -20°C until you are ready to proceed with the Second-Strand cDNA Synthesis and Amplification.

C. Second-Strand cDNA Synthesis and Amplification

1. To each first-strand synthesis reaction from Part B, add the following:

74 µl	RNase-free Water
10 µl	10X PCR Buffer
2 µl	dNTP Mix
2 µl	Full Spectrum PCR Primer
2 µl	PCR Polymerase
100 µl	Total volume (including the 10 µl from Part B)

2. Place the reactions in a thermal cycler, and cycle using the following program:

   - 95°C for 4 min
   - 68°C for 5 min

   - 95°C for 25 sec
   - 58°C for 1 min
   - 68°C for 1.5 min

   - (95°C for 25 sec; ***68°C for 1.5 min****) for 10+ cycles (see Note)
   - 68°C for 2 min
   - 15°C hold

   **** IMPORTANT: If your RNA sample is degraded, use an extension time of 1 minute in order to generate an optimal yield.***

   Note: You will need to vary the number of cycles depending on the amount of starting RNA. Refer to the table below to determine the approximate number of times you should cycle:

Starting RNA (ng)	**# Cycles ****
300	10
100	11
50	12
25	13
12	14
6	15

   ****** If your RNA sample is severely degraded, add 2 cycles.

3. After amplification, run 2.5 µl of each reaction on a 3% agarose gel in 1X TBE or TAE Buffer. Include a DNA size ladder with markers in the range of 50-2,000 bp (*e.g.*, Bio-Rad AmpliSize™ DNA Ladder). You should see results similar to those shown in Fig. 6. The typical yield from 50 ng RNA and above is 2-3 µg cDNA; from 5 ng, the yield is 1-1.5 µg.

Depending on your particular RNA sample, more cycles may be necessary. If so, perform an additional 2 cycles and check your RNA again. You do not need to add additional PCR Polymerase, even if your reaction was cycled overnight, as long as you held the reaction at 15°C after cycling. You can continue adding two-cycle increments until you see the sufficient product from your amplification reaction, however, you should not exceed 30 cycles.

The amplified cDNA may be stored at 4°C for a couple of weeks. For long-term storage, we recommend storing the cDNA at -20°C.

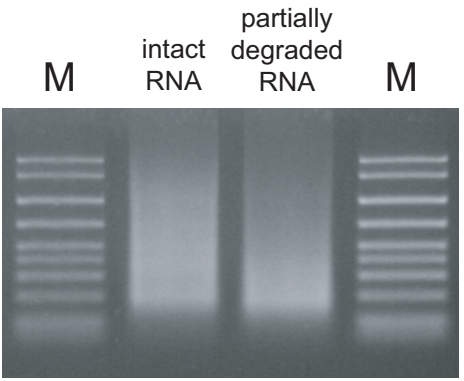

Fig. 6. Amplification Results. As shown when 2.5 µl of the amplified reaction with the Control RNA was run on a 3% agarose gel, the amplification process should generate a smear of cDNA with a size range from 150 bp to approximately 1,500 bp. The smear will look a little different depending on whether your starting RNA was mostly intact (middle left lane) or somewhat degraded (middle right lane). For intact starting RNA, the average size of the amplified cDNA should be approximately 500 bp. If you do not see this smear, refer to the Troubleshooting Section of this manual.
M: 100 bp DNA Ladder, 50 bp – 2,000 bp

D. Gene-Specific Amplification Using Amplified cDNA

The amplified cDNA is ready to use as a template without purification.

We recommend that the amplified cDNA be added as a 2% component of your gene-specific PCR reactions. Thus, for a 25 µl gene-specific PCR reaction, you should add 0.5 µl of the Full Spectrum™ amplified cDNA; for a 50 µl PCR reaction, add 1.0 µl of the amplified cDNA, etc.

For gene-specific PCR, we typically recommend using primers that are designed to amplify a region of 100-250 bp. Also, we recommend using a "hot start" PCR polymerase. For real-time Q-PCR, we suggest TaqMan® Assays.

III. Troubleshooting

A. No Product from cDNA Amplification

If, after step C.3, you do not see a smear on the 3% gel for your RNA samples, try the following suggestions:

If you do not see a smear for any samples, including the Control RNA…

- One or more of the reagents were omitted during the procedure or the volume of the reactions is incorrect. Calibrate your pipette and try amplifying the Control RNA again.

If you see a smear for the Control RNA, but not for your RNA samples…

- You may have less starting RNA than measured. Place the amplification reactions back in the thermocycler for an additional three cycles (95°C for 20 sec; 68°C for 1.5 min). *For degraded RNA, use an extension time of 1 min.* If the expected smear described in Section C.3 is generated, you should continue with qRT-PCR.
- If, after additional cycles, there is still no smear or a very weak smear compared with the Control RNA reaction, your RNA may either be (1) too degraded or (2) contain an inhibitor. Try the amplification again after repurifying the RNA. If you still do not get sufficient yield, try a different RNA purification kit.

B. No Product with Gene-Specific Primers

If you have confirmed that the amplification in step C.3 was successful, but you do not get product or get non-specific product with your gene specific primers, try the following suggestions:

If you do not get any product with your gene-specific primers…

- There may be a problem with your PCR reagents. Try to amplify with primers specific for an abundant gene such as GAPDH or β-actin. If the PCR still fails to generate a product after 25 cycles, try using new PCR reagents and enzyme.

If you see smears or non-specific products with your gene-specific primers…

- In general, we recommend using some form of "hot-start" PCR system to get specific priming.
- If you are using "hot start," and get smearing, you may be using too much amplified cDNA. Reduce the amount of the amplified cDNA you add to the gene-specific PCR by half and three-quarters. As noted, the volume of the amplified cDNA added to the gene-specific reaction should not exceed 2%.

IV. Appendix

A. Related Products

- **Full Spectrum™ MultiStart Primers for T7 IVT** (Cat. # RA300A-2)
  Extract more data from RNA than currently available primers in nearly all commercially-available T7 IVT kits using Full Spectrum™ technology. Just replace the existing T7 primer with the Full Spectrum™ primers. Compatible with Affymetrix GeneChip® hybridization.

- **Global MicroRNA Amplification Kit** (Cat. # RA400A-1)
  Simple amplification kit provides a quick and robust way to amplify cDNA for qRT-PCR and microarray studies from as little as 50 ng of starting total RNA.

- **MicroRNA Discovery™ Kit** (Cat. # RA410A-1)
  Uniformly and reproducibly amplify limited amounts of non-degraded as well as degraded RNA to provide sufficient template for the cloning of selected small RNA gene transcripts processed by RNase III. Amplification of novel microRNAs can be completed in less than 1 day, with 0.5 μg of total RNA.

B. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

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E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

V. Licensing and Warranty Statement

Limited Use License

Use of the Full Spectrum™ Complete Transcriptome RNA Amplification Kit (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

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SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

Limited Warranty

SBI warrants that the Product meets the specifications described in the accompanying Product Analysis Certificate. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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